

**Case No. 13-72346**

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**IN THE UNITED STATES COURT OF APPEALS  
FOR THE NINTH CIRCUIT**

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**POLLINATOR STEWARDSHIP COUNCIL, AMERICAN HONEY  
PRODUCERS ASSOCIATION, NATIONAL HONEY BEE ADVISORY  
BOARD, AMERICAN BEEKEEPING FEDERATION, THOMAS R. SMITH,  
BRET L. ADEE, and JEFFERY S. ANDERSON,**

Petitioners,

v.

**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*,**

Respondents,

and

**DOW AGROSCIENCES,**

Respondent-Intervenor.

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On Petition for Review of an Order of the  
United States Environmental Protection Agency

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**DECLARATIONS IN SUPPORT  
OF PETITIONERS' OPENING BRIEF**

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**TAB 1**

**UNITED STATES COURT OF APPEALS  
FOR THE NINTH CIRCUIT**

POLLINATOR STEWARDSHIP COUNCIL,	)	
et al.	)	Docket No. 13-72346
	)	
Petitioners,	)	
v.	)	
	)	
UNITED STATES ENVIRONMENTAL	)	
PROTECTION AGENCY, et al.	)	
	)	
Respondents,	)	
and	)	
	)	
DOW AGROSCIENCS LLC.	)	
	)	
Respondent-Intervenor.	)	
_____	)	

**DECLARATION OF BRET L. ADEE**

I, Bret L. Adee, declare as follows:

1. I am a resident of Bruce, South Dakota. I have personal knowledge of the matters stated herein and, if called as a witness, could and would competently testify thereto.

2. I am a third-generation commercial beekeeper and a co-owner, along with my father and brother, of Adee Honey Farms. Founded in 1957, Adee Honey Farms is today the nation’s largest beekeeping operation. We manage some 90,000 honeybee colonies and have about 50 full-time employees.

3. I am the president of the Pollinator Stewardship Council (“PSC”) and co-Chair of the National Honey Bee Advisory Board (“NHBAB”). I am also a member of the American Honey Producers Association, the South Dakota Beekeepers Association, and the California State Beekeepers Association.

4. PSC is a non-profit organization incorporated in Kansas in 2012. The mission of PSC is to defend managed and native pollinators vital to a sustainable and affordable food supply from the adverse impacts of pesticides. As pollination is required for one-third of the nation’s food supply, we accomplish our mission by: (1) ensuring that state agencies and the U.S. Environmental Protection Agency (“EPA”) enforce laws to protect pollinators from pesticides; (2) providing advocacy, guidance and tools for beekeepers to defend their bees from the detrimental effects of pesticides; and (3) raising awareness about the adverse impacts of pesticides on pollinators. Formerly known as the National Pollinator Defense Fund, the PSC board of directors adopted our new organizational name in October 2013 to better reflect the work of the organization.

5. NHBAB strives to promote honey bee sustainability through balanced pesticide policy, evidence-based decisions, and proactive education. Working collaboratively with other organizations, we seek to protect pollinators from the dangers of pesticides and to ensure that pollinator protection receives proper attention at a national policy level. NHBAB represents the two national beekeeper

trade associations in the U.S.: the American Beekeeping Federation and the American Honey Producers Association. I estimate that 80% of the professional beekeepers in the United States are members of ABF and/or AHPA.

6. The trade organizations and members that PSC and NHBAB were founded to protect and represent are extremely concerned about the adverse impact that pesticides, including sulfoxaflor and other neonicotinoids, are having on pollinators across the country. As a professional beekeeper, I share their concern.

7. At Adee Honey Farms, our bees risk exposure to pesticides in general, and sulfoxaflor in particular, at just about every point during the year. From May through September, we run our bees in Nebraska and South Dakota, where they inevitably are exposed to pesticides applied to sunflowers, canola, soybeans, corn, and other “bread basket” crops. Our bees are exposed to pesticides in multiple ways. Drift of pesticide from fields may come in direct contact with our bees or may affect areas where our bees are foraging. Sometimes crops or weeds within the treated fields themselves attract the bees. And for some crops, if they were treated with a systemic pesticide like sulfoxaflor, the pesticide may be in the plant itself, where the bee can be exposed by gathering contaminated pollen and nectar on that plant.

8. Bees customarily forage up to one mile from the hive depending on forage availability, but in lean times a bee might forage as far away as 5 miles. It

is not uncommon to find soybeans well within a mile of my hives and within exposure range for my bees in both South Dakota and Nebraska. In fact, it would be difficult for me to place my hives in those states in such a way as to fully avoid soybeans.

9. According to the States of South Dakota and Nebraska's official pesticide registration websites, sulfoxaflor was registered for soybean and barley use in South Dakota and Nebraska in May of 2013. Given the prevalence of soybeans in particular in these two states, it is impossible for me to place my bees in such a way as to avoid exposure to these crops or areas where these crops are grown. I am certain that my bees have therefore been exposed to and/or will be exposed to sulfoxaflor.

10. In October, we haul our bees to Kern County, California where we contract to provide pollination services primarily for almond, blueberry, and cherry growers. In California, we also run our bees in citrus orchards where sulfoxaflor has also been approved for use. We have, unfortunately, had to significantly cut back on our citrus contracts due to the damaging effects of pesticides in citrus groves. This resulted in a large drop in income from this work, and to eliminate citrus entirely would have yet another significant adverse effect on my business.

11. After the bloom in California, we will take some of our bees north to Washington to work the apple bloom before eventually returning to the Midwest.

According to the State of Washington official pesticide registration website, pesticide products containing sulfoxaflor are registered for use in Washington.

12. Like most beekeepers, Adee Honey Farms has experienced abnormally high incidences of hive failure in recent years. Prior to 2005, we would expect to lose between 3-8% of our colonies over the course of the winter. Now, we consider it a good year if we lose only 20%. In 2012, we lost 42% of our hives over winter, but by the time we came around to pollinate almonds in the early spring, our losses were at 55%. Our losses have increased at the same time that the number of registered neonicotinoid or similar-acting pesticides have increased, with sulfoxaflor being the most recent addition to the list.

13. In addition to the loss in income and additional costs associated with my bees' exposures to pesticides, my costs of keeping my bees alive has gone up. Before approximately 2005, I only provided food supplementation to my bees part of the time, usually in the winter when their natural food sources were low. Starting approximately 8 to 10 years ago, I started increasing supplement use and hitting my colonies hard with pollen substitutes in an effort to combat the losses and colony-weaknesses I was encountering. I am now providing pollen supplements to me bees in the fall – when they should be in excellent condition after a full summer – as well as in the winter. This is because they are now exposed to so many pesticides in the Dakotas and Nebraska in the summer and

sulfoxaflor will be an added burden on them. Because I am already using these kinds of tools to try and keep my colonies built up, I have no additional measures at my disposal to protect against the added exposures and weakening caused by sulfoxaflor.

14. Bee pollination is responsible for more than \$15 billion in increased crop value each year. About one mouthful in three in our diet directly or indirectly benefits from pollination. *See* Exh. A hereto. Commercial production of many crops like almonds and other tree nuts, berries, fruits and vegetables are dependent on pollination by honey bees. Almonds, for example, are completely dependent on honey bees for pollination. In California, the almond industry requires the use of 1.5 million colonies of honey bees, approximately 60 percent of all managed honey bee colonies in the United States. These foods give our diet critical diversity, flavor, and nutrition. EPA failed to consider these important benefits of bees to the food industry (as well as the economics of the beekeeping industry itself) when it claimed to examine the risks and benefits of registering sulfoxaflor.

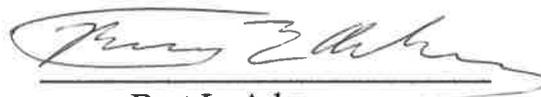
15. Like me, the vast majority of the individual members of PSC and NHBAB have suffered significant and increasing losses of colonies since the mid-2000s. Given that sulfoxaflor has been registered for use on multiple crops and the migratory nature of many commercial beekeeping operations, it is inevitable that most PSC and NHBAB members – like me – will suffer additional losses from

acute and chronic exposure to sulfoxaflor. Any additional losses will injure me economically.

16. Requiring EPA to reconsider the registration of sulfoxaflor and in doing so to properly assess the potential toxicity and impacts to bees, both acute and chronic and in combination with the many other chemical assaults to bees in the last 10 years, will begin to redress and hopefully reverse the damage from pesticide registrations that have been heedless or less than fully attentive to the serious economic impacts to the bee industry from neonicotinoids and systemic pesticides.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge and belief.

Dated: October 22, 2013



Bret L. Adce

# Exhibit A

To Declaration of Bret L. Adee

*Review*

# Importance of pollinators in changing landscapes for world crops

Alexandra-Maria Klein<sup>1,\*</sup>, Bernard E. Vaissière<sup>2</sup>, James H. Cane<sup>3</sup>,  
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and Teja Tscharntke<sup>1</sup>

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The extent of our reliance on animal pollination for world crop production for human food has not previously been evaluated and the previous estimates for countries or continents have seldom used primary data. In this review, we expand the previous estimates using novel primary data from 200 countries and found that fruit, vegetable or seed production from 87 of the leading global food crops is dependent upon animal pollination, while 28 crops do not rely upon animal pollination. However, global production volumes give a contrasting perspective, since 60% of global production comes from crops that do not depend on animal pollination, 35% from crops that depend on pollinators, and 5% are unevaluated. Using all crops traded on the world market and setting aside crops that are solely passively self-pollinated, wind-pollinated or parthenocarpic, we then evaluated the level of dependence on animal-mediated pollination for crops that are directly consumed by humans. We found that pollinators are essential for 13 crops, production is highly pollinator dependent for 30, moderately for 27, slightly for 21, unimportant for 7, and is of unknown significance for the remaining 9. We further evaluated whether local and landscape-wide management for natural pollination services could help to sustain crop diversity and production. Case studies for nine crops on four continents revealed that agricultural intensification jeopardizes wild bee communities and their stabilizing effect on pollination services at the landscape scale.

**Keywords:** agriculture; conservation; pollination; biodiversity; spatial ecology; wild bees

## 1. INTRODUCTION

Ecosystem services, defined as the benefits to human welfare provided by organisms interacting in ecosystems, are considered to be at risk (Daily 1997; Palmer *et al.* 2004). Pollination by wild animals is a key ecosystem service. Although crop pollination is commonly cited as an example of an endangered ecosystem service (Corbet 1991; Williams 1994; Ingram *et al.* 1996; Matheson *et al.* 1996; Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; Kevan & Phillips 2001; Steffan-Dewenter *et al.* 2005, but see Ghazoul 2005), detailed studies of the crop pollination systems are incomplete or out of date. Animal pollination is important to the sexual reproduction of many crops (McGregor 1976; Crane & Walker 1984; Free 1993; Williams 1994; Nabhan & Buchmann 1997; Westerkamp & Gottsberger 2000) and the majority of wild plants (Burd 1994; Kearns *et al.* 1998; Larson & Barrett 2000; Ashman *et al.* 2004), which can also be important for providing calories and micronutrients for

humans (Sundriyal & Sundriyal 2004). Furthermore, the decline of pollinating species can lead to a parallel decline of plant species (Biesmeijer *et al.* 2006).

For tropical crops, Roubik (1995) provided a detailed list for 1330 species and compiled a list of potential breeding systems and pollinating taxa. From this list, *ca* 70% of tropical crops seem to have at least one variety for which production is improved by animal pollination.

For European crops, Williams (1994) assessed the pollinator needs for 264 crop species and concluded that the production of 84% of these depends at least to some extent upon animal pollination. Previous estimates have used mostly secondary data and relied on crude guesses of the proportional contribution of pollinators to crop production. These rough estimates can be deceptive as they often neither consider variation in the level of dependence on animal pollination nor take into account the importance of the crop to consumers. The major caloric inputs in the human diet come from a few staple foods with large world production for which animal pollination is irrelevant (Richards 2001; Ghazoul 2005), or come indirectly via animals fed with these same staple crops.

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Some authors provide coefficients of dependence on animal-mediated pollination for several crops (Borneck & Merle 1989; Robinson *et al.* 1989<sub>a,b</sub>; Morse & Calderone 2000), but despite their continuing acceptance, most of these reports do not cite data sources, and so it is impossible to assess the reported level of dependence. Williams (1994) provided coefficients for the dependence of European crops on animal pollination and estimated the proportion of insect pollinators that are honeybees, using information from Crane & Walker (1984) and Free (1993). Both studies are less relevant today, because many new crop varieties and pollination studies are available. To adequately evaluate the importance of animal pollination for plant products in our food supply, and for economic analyses of crop pollination by animals, we need a global review of crops considering their breeding systems, their flower-visiting fauna and the level of production increase resulting from animal visitation and pollination, as supported by experimental evidence (Kevan & Phillips 2001).

Honeybees, mainly *Apis mellifera*, remain the most economically valuable pollinators of crop monocultures worldwide (McGregor 1976; Watanabe 1994; also shown for several single crops, e.g. Roubik 2002 for coffee in Panama) and yields of some fruit, seed and nut crops decrease by more than 90% without these pollinators (Southwick & Southwick 1992). When wild bees do not visit agricultural fields, managed honeybee hives are often the only solution for farmers to ensure crop pollination. Compared with the management of several wild bees, honeybees are versatile, cheap and convenient, but for some crops they are not the most effective pollinators on a per flower basis (reviewed in Parker *et al.* (1987), Torchio (1990), Richards (1996), Cane (1997<sub>a</sub>) and Westerkamp & Gottsberger (2000); see also Bosch & Blas (1994) for almond; Cane (1997<sub>b</sub>) and Javorek *et al.* (2002) for blueberry; Kremen *et al.* (2002, 2004) for watermelon; Klein *et al.* (2003<sub>a,b</sub>) for highland and lowland coffee; Cane (2005) for raspberry and blackberry; Greenleaf & Kremen (in press) for field tomatoes; Bosch *et al.* (2006) for cherry). Other crops await similar comparative pollinator study. The numbers of managed honeybee colonies are declining in some parts of the world (Williams *et al.* 1991; Matheson *et al.* 1996; Delaplane & Mayer 2000; Anonymous 2005) largely owing to: (i) the spread of pests like parasitic mites (*V. jacobsoni*, *V. destructor* and *Acarapis woodi*; Downey & Winston 2001; Chen *et al.* 2004), the small hive beetle (*Aethina tumida*; Evans *et al.* 2003) and the microsporidian parasite *Nosema ceranae* (Higes *et al.* 2006), (ii) improper pesticide and herbicide use (Ingram *et al.* 1996), (iii) ageing of the beekeeper population in Europe and North America, and (iv) lower market prices for their products and services. Indeed, declining honeybee availability led to recent concern over pollination shortfalls such as those seen for almonds in California ([www.almondboard.com](http://www.almondboard.com)). This situation also highlights the potential risk of our sole reliance on honeybees for agricultural pollination.

Fragmentation and degradation of near- and semi-natural habitats can be detrimental to bee communities (Rathcke & Jules 1994; Kremen *et al.* 2002, 2004; Steffan-Dewenter *et al.* 2002, 2006; Larsen *et al.* 2005; Cane *et al.* 2006). The main causal factor is loss or dissociation of important resources for food and nesting (Hines & Hendrix 2005; Potts *et al.* 2005). Conservation

of natural- and semi-natural habitats in agricultural landscapes to increase and protect bee's resources may be useful to improve pollination services. While landscape effects are known to affect communities of herbivorous and predatory/parasitic insects in agro-ecosystems (reviewed in Cronin & Reeve 2005; Tscharntke *et al.* 2005; Bianchi *et al.* 2006), a similar evaluation of landscape impact on crop pollination is lacking.

In this review, we summarize and evaluate information on three issues:

- (i) the identification of leading global crops that depend on animal pollination for their production and their level of dependence on pollinators,
- (ii) the influence of land-use changes at both local and landscape scales for pollinator communities and their services, and
- (iii) future options for landscape and agricultural management to enhance wild pollinators and ensure pollination services for crop production.

## 2. MATERIAL AND METHODS

We first estimated the proportion of crop production depending on animal pollination. We selected the leading global crops on the world market out of the FAO crop production list for the year 2004 (FAOSTAT 2005), such that the aggregate represented 99% of total global food production (figure 1). We chose single crops and commodities used for human food with an annual production of at least 4 000 000 Metric tonnes (Mt). Production values are listed individually for the single crops. Production of the commodity crops is pooled in not elsewhere specified (NES) commodities. A commodity is an aggregation of different crops (e.g. fresh vegetables NES includes 21 crops). Commodity compilation is based on a questionnaire that countries fill out to include important crops for the world market which are not listed as a single crop by the FAO. Fifty-seven leading single crops and five commodities (including 67 commodity crops) represented 99% (94.5 and 4.5%, respectively) of the total global food production.

Although production quantities for each commodity group are known, there is no breakdown for each commodity crop within these five groups, so we classified the annual production of the commodities with respect to its pollinator dependence as 'unknown'. We individually classified each of the resulting 124 crops (57 leading single and 67 leading commodity crops) into four categories of pollinator dependence:

- (i) production increase with pollinators for plant parts that we consume (we define production as increased fruit set, fruit weight and/or quality, and seed number and/or quality, when pollinators have access to the flowers in contrast to pollinator exclusion experiments),
- (ii) increase in seed production with pollinators to produce the vegetative parts that we consume,
- (iii) increase in seed production with pollinators for breeding alone, as the plants reproduce vegetatively and we consume the vegetative parts, and
- (iv) no production increase with pollinators.

We next assessed the level to which animal pollination matters to global crops directly used by humans. For this approach, we expanded our list using all the crops listed to be

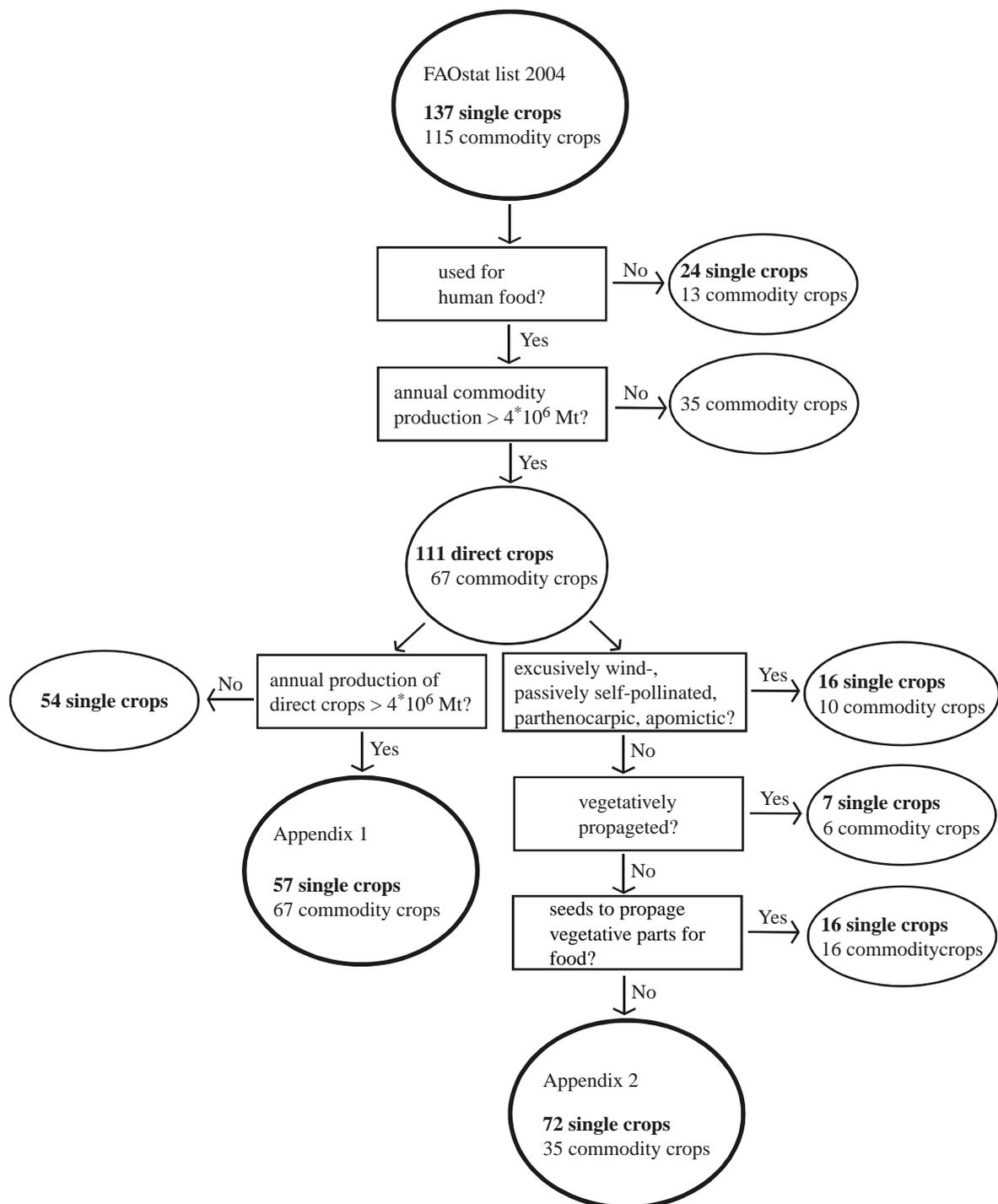


Figure 1. Crop selection pathway to estimate the annual world production that is influenced by animal pollination (electronic supplementary material 1; lower left side) and to evaluate the levels of dependence on animal pollination for crops important in the global market (electronic supplementary material 2; right side). Single crops are crops directly listed with their production by the FAO and commodity crops are combined to a commodity with an aggregated production value.

important on the world market, not restricted to the leading crops, as was the case for electronic supplementary material 1. We started with the same list used for electronic supplementary material 1, the complete set of 137 single crops and 5 commodities (93 commodity crops) listed by the FAO for the year 2004. We then reduced this list to 74 single crops and 33 commodity crops, a total of 107, following the pathway illustrated in figure 1.

Free (1993) summarized the key references for pollination requirements for 75 out of the 107 crops. We extended and updated his review, including both more recent literature and

earlier studies not cited in Free (1993). For each listed crop, we provide the following information:

- (i) Flower morphology and breeding system.
- (ii) Capacity of the crop to produce fruit and/or seeds without pollinators.
- (iii) Animal groups or species known to be important flower visitors or pollinators; the primary pollinating species are identified if there is a species for which at least 80% of their single flower visits result in a fruit (Klein *et al.* 2003a,b) or species that improve fruit and

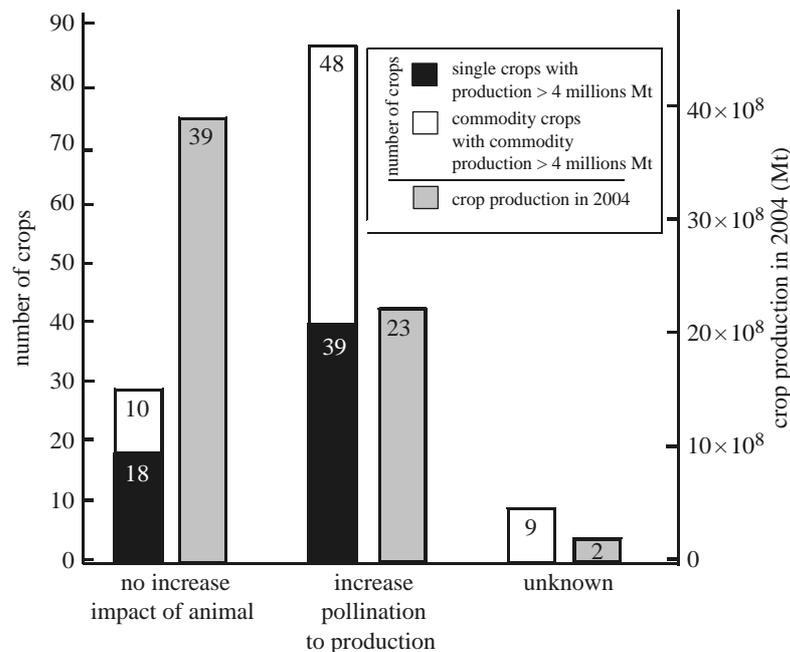


Figure 2. Relative importance of animal pollination for the leading global crops and commodities used for human food and selected by their annual production in 2004. We considered crops and commodities with an annual production greater than 4 000 000 Metric tonnes (Mt) as these comprise 99% of the 2004 total crop production listed for human food. The number of crops and the production are listed according to their production increase with pollinators (see electronic supplementary material 1 for details). Single crops and commodity crops in NES\* commodities are separated. The category 'unknown' includes only commodity crops for the number of crops while the 'unknown' production is the production of the leading commodities, as the production value of each commodity crop is not known. Crops in the 'increase' category could be classified into three sub-categories with the following number of species and total production figure for the individual crops: production increase with pollinators for plant parts that we consume (fruits and/or seeds: 26 crops with  $12\ 108\ \text{Mt} = 55\%$ ); increase in seed production with pollinators to produce the vegetative parts that we consume (six crops with  $2108\ \text{Mt} = 9\%$ ); and increase in seed production with animals for breeding alone, as the plants reproduce vegetatively and we consume the vegetative parts (seven crops with  $8108\ \text{Mt} = 36\%$ ). NES\* is an abbreviation for not elsewhere specified; leading commodities are fresh vegetables NES, fresh fruits NES, fresh tropical fruits NES, roots and tubers NES and pulses NES. Commodity crops are included based on a questionnaire that countries fill out to include important crops for the world market which are not listed as single crops.

seed quality and quantity when abundant as compared with the level when all flower visitors are excluded.

- (iv) Magnitude of the improvement in production and quality when pollinated by animals. We scored the degree of production dependence into five classes: (i) essential (production reduction by 90% or more without flower visitors), meaning that production requires animal pollination, (ii) high (40 to less than 90% reduction), (iii) modest (10 to less than 40%), (iv) little (greater than 0 to less than 10%), (v) no reduction, and (vi) unknown, meaning that no literature was available to adequately review the breeding systems or draw conclusions about pollinator dependence.

### 3. RESULTS AND DISCUSSION

#### (a) Importance of animal pollination for global crop production

Production of 39 of the leading 57 single crops increases with pollinating animals (figure 2). In aggregate, these crops account for 35% ( $23 \times 10^8\ \text{Mt}$ ) of global food production (figure 2), but because most of these crops are not entirely dependent on animal pollination, the amount of production directly attributable to animals is lower than this value. In addition, production of 48 of the 67 crops of the five leading global commodities increases with pollinating animals (figure 1). Only insects are demonstrated pollinators of the single crops, while

vertebrates pollinate very few commodity crops (e.g. feijoa is pollinated by birds and durian seems to be pollinated by bats, electronic supplementary material 2). Among the 57 single crops that show increased production, 26 (55% with  $12 \times 10^8\ \text{Mt}$  or 19% of global food) increase seed production with animal pollination to produce vegetative parts for human food, while an additional seven crops ( $8 \times 10^8\ \text{Mt}$ , 36%) show increased seed production for breeding alone, as the plants reproduce vegetatively and only vegetative parts are consumed (e.g. potatoes, sweet potatoes and manioc, electronic supplementary material 1). The production increase with pollinators for seeds of vegetatively propagated crops permits breeding progress and hybridization for the development of new varieties.

Animal pollination is irrelevant to 18 of the leading single crops (comprising 60% or  $39 \times 10^8\ \text{Mt}$  of the world production) and 10 of the leading commodity crops. These are wind- or passively self-pollinated grasses (cereals and sugarcane), dominating the leading global crop list (electronic supplementary material 1; figure 2).

Twenty per cent of the overall crop production comes from crops that increase fruit and vegetable production with animal pollination, and *ca* 15% comes from crops that increase seed production with animal pollination. Our results further show that a majority of global crops could experience production loss owing to pollinator limitation (39 single crops increase fruit, vegetable or seed production with pollinators compared with 18 that do not, and 87 of

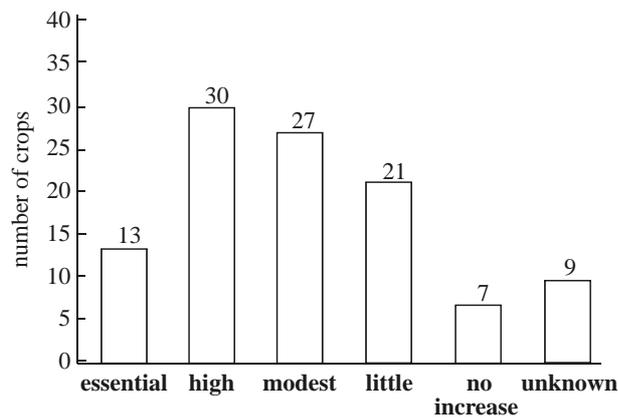


Figure 3. Level of dependence on animal-mediated pollination. The selected crops are those included directly in the production list published by the FAO for 2004 (FAOSTAT 2005). We further included commodity crops for which the production was pooled in commodities with an annual 2004 commodity production greater than 4 000 000 Metric tonnes (Mt). Only crops that produce fruits or seeds for direct human use as food were considered. We did not include: (i) crops for which seeds are only used for breeding or to grow vegetable parts for direct human use or for forage, and (ii) crops known to be only wind-pollinated, passively self-pollinated or reproduced vegetatively. **Essential**, pollinators essential for most varieties (production reduction by 90% more, comparing experiments with and without animal pollinators); **high**, animal pollinators are extreme (40 to less than 90% reduction); **modest**, animal pollinators are clearly beneficial (10 to less than 40% reduction); **little**, some evidence suggests that animal pollinators are beneficial (greater than 0 to less than 10% reduction); **no increase**, no production increase with animal-mediated pollination; **unknown**, empirical studies are missing.

the commodity crops increase production compared with 28 that do not; figure 2). Included are many fruit crops that provide essential macro- and micronutrients contributing to a healthy diet. These results support the contention of Richards (2001) and Ghazoul (2005) that primary food production, and especially our staple foods, is independent of insect pollination. Thinking beyond caloric intake, however, our results support the opinion of Steffan-Dewenter *et al.* (2005) that our diet would be greatly impoverished, both nutritionally and culturally, if pollination services further decline.

In a second list (electronic supplementary material 2), we quantified the level of dependence on animal pollination. We found empirical evidence for increased production with pollinators in 92 out of 108 selected crops (figure 3). Among these 92 crops, for the majority (82 crops), data were available from experiments comparing measures of pollination (e.g. fruit set, number of seeds, fruit or seed weight, or pollen deposition) at the level of flowers, inflorescences or whole plants, with and without access to pollinators. For 10 crops, we classified the evidence for increased production with pollinators as 'indirect evidence', because experiments with pollinator exclusion were lacking, but the experiments demonstrated, for example, self-incompatibility and a need for cross pollination that could not be achieved by wind (electronic supplementary material 2; figure 3). Animal pollination was found to be essential for most varieties of the following 13 crops: atemoya, Brazil nut, cantaloupe, cocoa, kiwi, macadamia nut, passion fruit, pawpaw (Indian banana),

rowanbarry, sapodilla, squashes and pumpkins, vanilla and watermelon. An additional 30 crops showed increased fruit and/or seed production for most species and varieties with animal pollination. Twenty-seven crops show a modest increase in production, and for 21 crops, production of some species or varieties increase little, others not at all. For seven crops, production did not increase in the studies available: chick pea, garden and field peas and lentil, which are passively self-pollinated, and olive, pepper, quinoa and grapes, which rely on passive self- and wind-pollination. Pollination needs of nine crops remain unknown (figure 3; electronic supplementary material 2).

Gaps in our knowledge of pollination requirements are illustrated by the example of highland coffee, one of the better studied crops. Although the breeding systems are well studied and pollinators have been identified in different coffee production regions, few varieties have been studied, and production of some varieties may not increase with animal pollination as much as those studied to date (A.-M. Klein, unpublished data). The need to consider different genetic materials is also highlighted by the fact that varieties of many crops, such as citrus, blueberries, most stone fruit crops, and almonds, show great production variation with animal pollination (see Ortega *et al.* 2002 for almond). We also do not know much about the mechanisms of pollination provided by most pollinator species (Klein *et al.* 2003a), and flower-visiting insect communities of different production regions across the world can differ greatly. For example, the flower visitors to coffee in Ecuador with more than 95% social and less than 5% solitary bees (Veddeler *et al.* 2006) are very different from flower-visiting communities in Indonesia with 70% social and *ca* 30% solitary bees (Klein *et al.* 2003a,b). Such differences may lead to differences in pollination success.

#### (b) *Consequences of agricultural management at local and landscape scales for wild versus managed pollinators*

Wild bees and other insects can pollinate many crops, but their value for crop pollination has been overlooked for centuries. As their services are increasingly being recognized for agriculture (e.g. O'Toole 1993; Cane 1997b; Kevan & Phillips 2001; Klein *et al.* 2003a; Slaa *et al.* 2006), the adequate management of local agro-ecosystems and the conservation of suitable natural or semi-natural pollinator habitats in the surrounding landscapes are receiving more attention. Little information exists on the ways in which local management influences agricultural pollination (Richards 2001). Considering the 107 crops listed in electronic supplementary material 2, we found increased production with animal pollination of at least 10% or higher (categories essential, great and modest) for 63 crops, when considering only the crops for which field experiments were available ( $N=93$ ). Therefore, we suggest that pollination of at least these 63 crops should be vulnerable to agricultural intensification that may reduce the diversity and abundance of pollinators (e.g. Kremen *et al.* 2002; Klein *et al.* 2003a,b). Among the 63 crops, the production of 13 crops that are entirely dependent on pollinators to set fruits might be severely impacted by pollinator loss through agricultural intensification. This risk is the greatest for crops that rely on a narrow range of pollinating species, such as passion fruit and vanilla.

Table 1. Pollinator and pollination limitation in crop plants in response to land-use and landscape changes. (Significance \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .)

species name (common crop name)	land-use and landscape variable	pollination variable and significance level of reduction	reference
<i>Annona squamosa</i> × <i>A. cherimola</i> (sugar apple)	comparison of sites near and far from forest fragments	pollinator diversity*** (fruit set reduction with pollinator exclusion***)	Blanche & Cunningham (2005)
<i>Brassica napus</i> and <i>B. rapa</i> (turnip rape, canola and oilseed rape)	comparison of organic, conventional and geneti- cally modified (GM) fields	number of seeds per silique from a flower sample***	Morandin & Winston (2005)
	proportional area of unculti- vated land around fields within a 750 m radius	number of seeds per silique from a flower sample*	Morandin & Winston (2006)
<i>Citrullus lanatus</i> (watermelon)	comparison of organic versus conventional fields	number of pollen grains/ stigma, n.s.	Kremen <i>et al.</i> (2002, 2004)
	proportional area of oak woodland and chaparral habitat	number of pollen grains/ stigma***	Kremen <i>et al.</i> (2002, 2004)
<i>Citrus paradisi</i> (grapefruit)	distance from forest	number of pollen grains/ stigma* number of pollen tubes/stigma*	Chacoff (2006) and Chacoff & Aizen (2006)
<i>Coffea arabica</i> (coffee)	coffee plants near, intermedi- ate and far from forest fragments	number of pollen grains/ stigma***, fruit set*, seed mass**	Ricketts (2004) and Ricketts <i>et al.</i> (2004)
	distance from forest	fruit set**	Klein <i>et al.</i> (2003a)
	plant diversity	fruit set**	Klein <i>et al.</i> (2003a)
	coffee monocultures versus agroforestry	fruit set*	De Marco & Coelho (2004)
<i>Coffea canephora</i> (coffee)	comparison sites near and far from forest fragments	fruit set*	De Marco & Coelho (2004)
	distance from forest	fruit set**	Klein <i>et al.</i> (2003b)
<i>Dimocarpus longan</i> (longan fruit)	comparison sites near and far from forest fragments	number of fruits per centi- metre panicle*	Blanche <i>et al.</i> (in press)
<i>Helianthus annuus</i> (sunflower)	proportional area of natural habitat	wild bee diversity and abundance*** (estimated increase in seed set via single visit studies)	Greenleaf & Kremen (2006)
	organic versus conventional farm management	wild bee diversity and abun- dance, n.s.	Greenleaf & Kremen (2006)
<i>Lycopersicon esculentum</i> (tomato)	distance to natural habitat	<i>Bombus vosnesenskii</i> abundance***; <i>Anthophora</i> <i>urbana</i> abundance, n.s. (fruit set and fruit weight reduction with pollinator exclusion for variety with exserted stigma)	Greenleaf & Kremen (in press)
	percentage of eucalyptus forest surrounding orchards	<i>Trigona</i> abundance (seed set reduction with pollinator exclusion* and only <i>Trigona</i> pollinated*)	Heard (1994) and Heard & Exley (1994)
<i>Macadamia integrifolia</i> (maca- damia nut)	percentage of eucalyptus forest surrounding orchards	<i>Trigona</i> abundance (seed set reduction with pollinator exclusion* and only <i>Trigona</i> pollinated*)	Heard (1994) and Heard & Exley (1994)
	comparison of sites near and far from forest fragments	number of fruits/raceme*	Blanche <i>et al.</i> (in press)

We found 16 studies on the effects of agricultural intensification on pollination at local or landscape scale of nine crops on four continents (table 1). All of these studies show negative consequences of local and/or regional agricultural intensification for pollination. For watermelon and coffee, higher variation in pollination success was found in sites of intensified agriculture isolated from natural or semi-natural habitats (Kremen *et al.* 2004; Steffan-Dewenter *et al.* 2006).

The existing studies suggest that crops having a production increase with pollinators of at least 10% might show reduced fruit set and increased variance in fruit set at locations increasingly isolated from near-natural

habitats (figure 4). The impact of landscape context on visitation rates and fruit set of crops has been assessed as the proportion of near-natural habitats in the surrounding landscape (e.g. Kremen *et al.* 2004; Morandin & Winston 2006) or as the linear isolation distance from near-natural habitat (e.g. Klein *et al.* 2003a,b; Chacoff & Aizen 2006). We found a linear positive relationship between fruit set stability and isolation to the rainforest margin for lowland and highland coffee (Klein *et al.* 2003a,b), whereas a log-linear relationship was found for watermelons (Kremen *et al.* 2004). Agro-ecosystems with more semi-natural habitats are often more pollinator-species rich (Steffan-Dewenter *et al.* 2002; Kremen & Chaplin 2006;

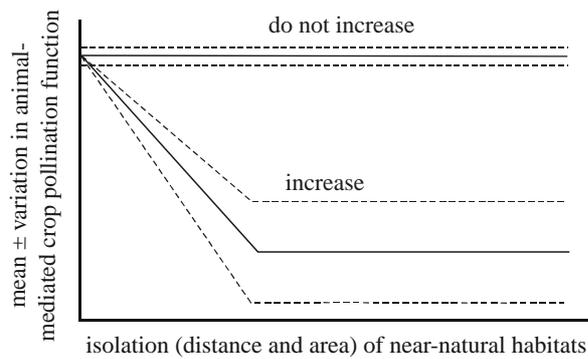


Figure 4. Expected relationship between the loss of animal-mediated crop pollination function (pollination variable usually measured as fruit or seed set in pollination studies and the variation usually measured as the coefficient of variation in the number or yield of fruits indicating crop production stability) and the effect of isolation from near-natural habitats (which means the area and distance of the main nesting and foraging habitats for the pollinators). Expected relationships in the absence of pollinator introduction are given for crops which are independent of animal pollination and for crops depending on animal pollination. Mean, solid line; variation, dashed line.

Steffan-Dewenter *et al.* 2006). There might be a threshold level of diversity necessary to maintain lower variation or higher stability in pollination. The exact shape of the function will depend on the biology of crop, crop variety, pattern of the landscape and regional pollinator community, but the available data indicate that pollination stability will increase in landscapes with a diverse and abundant pollinator community. The positive pollination effect on crop yield can however be reduced or hidden when other factors affecting crop yield, such as soil nutrients, microclimate, water, pest or disease status are suboptimal. Further, agricultural land use is not always expected to reduce pollination services. Some wild bees may benefit from agriculture, such as ground-nesting bees that use disturbed areas for nesting, or pollinators may benefit from pollen-rich crop fields, such as oilseed rape (Westphal *et al.* 2003), or from ecosystems in which agricultural areas provide a greater diversity, continuity or abundance of floral resources than original habitat types (e.g. Winfree *et al.* in press). Therefore, knowledge of the pollinator's resources and life-history traits is required to correctly predict the likely pollination responses (Cane *et al.* 2006). Failure of wild pollinators can be overcome by the provision of commercially managed bees, where they are effective and manageable pollinators available (Kremen *et al.* 2002), but this service generally comes at a cost. Finally, crops with little or no dependence on animal pollination will exhibit no relationship between pollination rates and isolation (figure 4).

Unfortunately, none of the landscape studies have been conducted over enough years to reliably estimate temporal variability in pollination. In some studies, samples were taken in two consecutive seasons (Kremen *et al.* 2002, 2004; Ricketts 2004; Ricketts *et al.* 2004), but a majority were carried out over only one season.

Studies that compare fruit or seed set of flowers in treatments with and without access by wild-pollinating species or with additional hand-pollination provide important data to identify key pollinating species (Canto-Aguilar & Parra-Tabla 2000; Javorek *et al.* 2002;

Cane & Schiffhauer 2003; Klein *et al.* 2003a,b; Greenleaf & Kremen 2006, in press; Blanche *et al.* in press), but few such studies are yet available. In spite of this information shortage, many reviews mention the neglected potential of wild bee species for crop pollination (O'Toole 1993; Corbet 1996; Williams 1996; Westerkamp & Gottsberger 2000; Goulson 2003). Buchmann & Nabhan (1996) suggested that *ca* 80% of the 100 most important staple crops (Prescott-Allen & Prescott-Allen 1990) are pollinated by wild insects. We found evidence for only 24 out of the 57 leading crops (42%) being pollinated by at least one wild bee species. We identified 57 species (mainly bees and only two vertebrate species) as not only flower visitors, but also true pollinators for the 107 global crops for direct human use (electronic supplementary material 2; table 2). Considering these 107 crops, empirical evidence with direct testing revealed that both honeybees (which can be managed or feral) and wild pollinators are valuable pollinators for 35 crops. For 12 crops, empirical studies provided evidence only for honeybees contributing to successful pollination, with wild pollinators mentioned as pollinators for 10 of these 12 crops, but without empirical data. For those cases where there was evidence for honeybees but not wild bees, the problem was generally a shortage of evidence, rather than evidence that wild bees were in fact poor pollinators. For nine crops, empirical studies showed evidence that wild pollinators contributed to successful pollination without similar evidence for honeybees, and for six (atemoya, cocoa, fig, passion fruit, oil palm and sapodilla) of these nine crops honeybees were not mentioned as pollinators. These nine crops depend strictly on, or production increased greatly with, wild pollinators, and interestingly, three of these crops—atemoya, passion fruit and vanilla—are produced by hand-pollination in many parts of the world, showing the severe lack of wild pollinators.

In most environments, both wild pollinators and honeybees will exploit flowers of crop species. For example, males of wild bees searching for mates disturbed honeybees during foraging, so that honeybees switched more often between lines of hybrid sunflower, and carried more pollen, thereby increasing the overall pollination service (Degrandi-Hoffmann & Watkins 2000; Greenleaf & Kremen 2006). Strawberry flowers visited by both wild and honeybees are more likely to be completely developed in contrast to flowers that are visited by only honeybees or only wild bees that tended to have misshapen fruits (Chagnon *et al.* 1993). Effects such as this have rarely been looked for, but may prove to be widespread.

#### 4. MANAGEMENT CONCLUSIONS AND FUTURE DIRECTIONS

##### (a) Pollinator management

Populations of wild pollinators can enhance production of some crops and are, in this way, an important natural resource; but populations of wild pollinators are frequently too sparse to adequately pollinate crops in agriculturally intensive environments (table 1). The landscape studies summarized in this review were all published during the last 5 years. Although more research is needed on a landscape scale, we are in a much better position today than we have been in the past to recommend landscape management practices to enhance wild pollinators. We

Table 2. Species list of known pollinators for global crops that are grown for direct human consumption.

pollinator group	species
honey bees	<i>Apis cerana</i> Fabr., <i>A. dorsata</i> Fabr., <i>A. florea</i> Fabr. and <i>A. mellifera</i> L.
stingless bees	<i>Melipona favosa</i> Fabr., <i>M. subnitida</i> Ducke, <i>M. quadrifasciata</i> Lepeletier, <i>Nanotrigona perilampoides</i> Cresson, <i>N. testaceicornis</i> Lepeletier, <i>Trigona cupira</i> Sm., <i>T. iridipennis</i> Smith, <i>T. (Lepidotrigona) terminata</i> Smith, <i>T. (Tetragonoula) minangkabau</i> Sakagami, <i>T. toracica</i> Smith and <i>Scaptotrigona depilis</i> Moure
bumble bees	<i>Bombus affinis</i> Cresson, <i>B. californicus</i> F. Smith, <i>B. hortorum</i> L., <i>B. hypnorum</i> L., <i>B. impatiens</i> Cresson, <i>B. lapidarius</i> L., <i>B. (Thoracobombus) pascuorum</i> Scop., <i>B. sonorus</i> L., <i>B. terrestris</i> L. and <i>B. vosnesenskii</i> Radoszkowski
solitary bees	<i>Amegilla chlorocyanea</i> Cockerell, <i>A. (Zonamegilla) holmesi</i> Rayment, <i>Andrena ilerda</i> Cam., <i>Anthophora pilipes</i> Fabr., <i>Centris tarsata</i> Smith, <i>Creightonella frontalis</i> Fabr., <i>Habropoda laboriosa</i> Fabr., <i>Halictus tripartitus</i> Cockerell, <i>Megachile (Delomegachile) addenda</i> Cresson, <i>M. rotundata</i> Fabr., <i>Osmia aglaia</i> Sandhouse, <i>O. cornifrons</i> Radoszkowski, <i>O. cornuta</i> Latreille, <i>O. lignaria lignaria</i> Say, <i>O. lignaria propinqua</i> Cresson, <i>O. ribifloris</i> Cockerell, <i>Peponapis limitaris</i> Cockerell, <i>P. pruinosa</i> Say, <i>Pithitis smaragdula</i> Fabr., <i>Xylocopa (Zonohirsuta) dejeanii</i> Lepeletier, <i>Xylocopa frontalis</i> Oliver and <i>Xylocopa suspecta</i> Moure
wasps	<i>Blastophaga psenes</i> L.
hover flies and other flies	<i>Eristalis cerealis</i> Fabr., <i>E. tenax</i> L. and <i>Trichometallea pollinosa</i> Townsend
beetles	<i>Carpophilus hemipterus</i> L. and <i>Carpophilus mutilatus</i> Erichson
thrips	<i>Thrips hawaiiensis</i> Morgan and <i>Haplothrips (Haplothrips) tenuipennis</i> Bagnall
birds	<i>Turdus merula</i> L. and <i>Acridotheres tristis</i> L.

need landscape management practices that boost native pollinator densities by increasing habitat-carrying capacity. We suggest integrating the following general practices into management plans: (i) increase nesting opportunities with the particular nesting needs of different pollinating species in mind and these may include gaps in surface vegetation or modifying cultivation practices (Shuler *et al.* 2005), retaining neighbouring forest nesting sites for ground-nesting bees (Cane 1997a,b) or leaving dead wood providing holes for cavity-nesting bees (Westrich 1996), (ii) increase forage by providing suitable diverse floral resources in the local area and the broader landscape during the season of pollinator activity (Kevan *et al.* 1990; Banaszak 1992; Westrich 1996; Goulson 2003; Ghazoul 2006). Crop rotation using these flowering plants should be especially applied in intensified uniform agricultural landscapes and may also help to enhance other ecosystem services such as soil improvement, pest management by breaking cycles of damaging pests or erosion control, (iii) enhance opportunities for colonization by connecting habitats with flowering strips and hedgerows around arable fields, small forest patches or even single trees as 'stepping stones' (Steffan-Dewenter *et al.* 2002, 2006; Pywell *et al.* 2006), and (iv) reduce the risk of population crashes in the field and the surrounding habitats by foregoing use of broad-spectrum insecticides during bloom, especially those with systemic or micro-encapsulated formulations that can contaminate nectar and pollen (Kevan 1975; Wood 1979; Delaplane & Mayer 2000). Financial burdens of these recommendations could be ameliorated through agro-environmental schemes, such as those in Europe and the United States, which compensate farmers who apply management strategies to conserve biodiversity.

### (b) Research needs

In this review, we found that inadequate information is available on the pollination biology and pollinator requirements of many crops, especially when considering differences among modern varieties and the contribution to pollination services by different pollinator species.

We need to assess the potential impact of pollinator loss for a given crop in a given production area. For this, we need to collect the following data: experimental fruit and seed set from flowers visited by animal pollinators versus unvisited flowers and those receiving airborne pollen flow or any passive self-pollination. As plants are often resource limited, treatments should ideally be applied to entire plants and not just a few flowers or a single branch, otherwise, extrapolation can overestimate pollen limitation (Ashman *et al.* 2004; Knight *et al.* 2006). Multi-year data are valuable as periodic weather perturbations are the norm and perennial plants tend towards alternate year of fruit and seed production (e.g. Herrera *et al.* 1998; Pías & Guitián 2006). Studies over multiple seasons are also necessary to truly understand the stability of the pollination service, because insect communities often show high temporal variation (Cane & Payne 1993; Roubik 2001) and habitat-specific temporal species turnover (Williams *et al.* 2001; Cane *et al.* 2005; Tylanakis *et al.* 2005).

Studies for only three crops (watermelon, highland- and lowland coffee) are available to address the links between a landscape variable and the stability of crop pollination. More research of this kind is needed. The list of pollinators known to be important for global crops was only 57 species, mainly bees. We found only one study showing birds to be effective pollinators on feijoa (Stewart 1989). We still need experiments to determine to what extent non-insects (birds, bats and other vertebrates) contribute to crop production. In addition, to adequately judge the value of conserving and managing for wild pollinators, key pollinators in the main producing areas must be identified, their habitat requirements studied and the economic benefit of their presence estimated (e.g. Cane 1997b; Larsen *et al.* 2005). Today, only few areas and crops have all the necessary data elements to assess the impact of pollinator loss.

Our four general recommendations for landscape management (nesting opportunities, floral resources, habitat connectivity and reduction of pesticides) can be applied to all crops dependent on animal pollination in all

production areas. For further specific recommendations, we emphasize the need to monitor the effects of applied management practices on crop production and stability in restoration programmes (e.g. Pywell *et al.* (2006) for pollinator foraging resources and Albrecht *et al.* in press for the pollination of three herb species). We also emphasize the collection of data for understanding the effects of spatial and temporal pollinator resource availability and for interaction effects between honeybees and other bee species for crop pollination to recommend future management applications.

Therefore, we urgently need more research in crop pollination along with better coordination of the research efforts at the community level in different producing areas to help sustain production of the diverse crops that nourish humanity.

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## REFERENCES

- Albrecht, M., Duelli, P., Müller, C. B., Kleijn, D. & Schmid, B. In press. Swiss agri-environment scheme enhances pollinator diversity and plant reproductive success in nearby intensively managed farmland. *J. Appl. Ecol.*
- Allen-Wardell, G. *et al.* 1998 The potential consequences of pollinator declines on the conservation of biodiversity and stability of crop yields. *Conserv. Biol.* **12**, 8–17. (doi:10.1046/j.1523-1739.1998.97154.x)
- Anonymous 2005 Audit de la filière miel août 2005. *Abeille de France* **919**, 479–496.
- Ashman, T. L. *et al.* 2004 Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**, 2408–2421.
- Banaszak, J. 1992 Strategy for conservation of wild bees in an agricultural landscape. *Agric. Ecosyst. Environ.* **40**, 179–192. (doi:10.1016/0167-8809(92)90091-O)
- Bianchi, F. J. J. A., Booij, C. J. H. & Tscharntke, T. 2006 Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proc. R. Soc. B* **273**, 1715–1727. (doi:10.1098/rspb.2006.3530)
- Biesmeijer, J. C. *et al.* 2006 Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* **313**, 351–354. (doi:10.1126/science.1127863)
- Blanche, R. & Cunningham, S. A. 2005 Rain forest provides pollinating beetles for atemoya crops. *J. Econ. Entomol.* **98**, 1193–1201.
- Blanche, K. R., Ludwig, J. A. & Cunningham, S. A. In press. Proximity to rainforest enhances pollination and fruit set in macadamia and longan orchards in north Queensland, Australia. *J. Appl. Ecol.* (doi:10.1111/j.1365-2664.2006.01234.x)
- Borneck, R. & Merle, B. 1989 Essai d'une évaluation de l'incidence économique de l'abeille pollinisatrice dans l'agriculture Européenne. *Apiacta* **XXIV**, 33–38.
- Bosch, J. & Blas, M. 1994 Foraging behaviour and pollinating efficiency of *Osmia cornuta* and *Apis mellifera* on almond (Hymenoptera, Megachilidae and Apidae). *Appl. Entomol. Zool.* **29**, 1–9.
- Bosch, J., Kemp, W. P. & Trostle, G. E. 2006 Bee population returns and cherry yields in an orchard pollinated with *Osmia lignaria* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* **99**, 408–413.
- Buchmann, S. L. & Nabhan, G. P. 1996 *The forgotten pollinators*. Washington, DC: Island Press.
- Burd, M. 1994 Bateman's principle and reproduction: the role of pollinator limitation in fruit and seed set. *Bot. Rev.* **60**, 83–139.
- Cane, J. H. 1997a Ground-nesting bees: the neglected pollinator resource for agriculture. *Acta Hort.* **437**, 309–324.
- Cane, J. H. 1997b Lifetime monetary value of individual pollinators: the bee *Habropoda laboriosa* at rabbiteye blueberry (*Vaccinium ashei* Reade). *Acta Hort.* **446**, 67–70.
- Cane, J. H. 2005 Pollination potential of the bee *Osmia aglaia* for cultivated red raspberries and blackberries (Rubus: Rosaceae). *Hortscience* **40**, 1705–1708.
- Cane, J. H. & Payne, J. A. 1993 Regional, annual and seasonal variation in pollinator guilds — intrinsic traits of bees (Hymenoptera, Apoidea) underlie their patterns of abundance at *Vaccinium ashei* (Ericaceae). *Ann. Entomol. Soc. Am.* **86**, 577–588.
- Cane, J. H. & Schiffhauer, D. 2003 Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon* [Ericaceae]). *Am. J. Bot.* **90**, 1425–1432.
- Cane, J. H., Minckley, R., Kervin, L. & Roulston, T. 2005 Temporally persistent patterns of incidence and abundance in a pollinator guild at annual and decadal scales: the bees of *Larrea tridentata*. *Biol. J. Linn. Soc.* **85**, 319–329. (doi:10.1111/j.1095-8312.2005.00502.x)
- Cane, J. H., Minckley, R., Roulston, T., Kervin, L. & Williams, N. M. 2006 Multiple response of desert bee guild (Hymenoptera: Apiformes) to urban habitat fragmentation. *Ecol. Appl.* **16**, 632–644.
- Canto-Aguilar, A. & Parra-Tabla, V. 2000 Importance of conserving alternative pollinators: assessing the pollination efficiency of the squash bee, *Peponapis limitaris* in *Cucurbita moschata* (Cucurbitaceae). *J. Ins. Conserv.* **4**, 203–210.
- Chacoff, N. P. In press. Los ecosistemas naturales como fuente de polinizadores para cultivos en el pedemonte de las yungas. Ph.D. thesis. Universidad Nacional del Comahue, Argentina.
- Chacoff, N. P. & Aizen, M. A. 2006 Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. *J. Appl. Ecol.* **43**, 18–27. (doi:10.1111/j.1365-2664.2005.01116.x)
- Chagnon, M., Gingras, J. & De Oliveira, D. 1993 Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). *J. Econ. Entomol.* **86**, 416–420.
- Chen, Y., Pettis, J. S., Evans, J. D., Kramer, M. & Feldlaufer, M. F. 2004 Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. *Apidologie* **35**, 441–448. (doi:10.1051/apido:2004031)
- Corbet, S. A. 1991 Bees and the pollination of crops and wild flowers in the European community. *Bee World* **72**, 47–59.
- Corbet, S. A. 1996 Which bees do plants need? In *The conservation of bees* (ed. A. Matheson, S. L. Buchmann, C. O'Toole, P. Westrich & J. H. Williams), pp. 105–114. London, UK: Academic Press.
- Crane, E. & Walker, P. 1984 *Pollination directory for world crops*. Bucks, UK: International Bee Research Association.
- Cronin, J. T. & Reeve, J. D. 2005 Host-parasitoid spatial ecology: a plea for a landscape-level synthesis. *Proc. R. Soc. B* **272**, 2225–2235. (doi:10.1098/rspb.2005.3286)

- Daily, G. C. 1997 *Nature's services: societal dependence on natural ecosystems*. Washington, DC: Island Press.
- Degrandi-Hoffmann, G. & Watkins, J. C. 2000 The influence that honey bees and wild bees foraging together have on sunflower cross-pollination and seed set. *Am. Bee J.* **137**, 565–566.
- Delaplane, K. S. & Mayer, D. F. 2000 *Crop pollination by bees*. New York, NY: CABI Publishing.
- De Marco, P. & Coelho, F. M. 2004 Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodivers. Conserv.* **13**, 1245–1255. (doi:10.1023/B:BIOC.0000019402.51193.e8)
- Downey, D. L. & Winston, M. L. 2001 Honey bee colony mortality and productivity with single and dual infestations of parasitic mite species. *Apidologie* **32**, 567–575. (doi:10.1051/apido:2001144)
- Evans, J. D., Pettis, J. S., Hood, W. M. & Shimanuki, H. 2003 Tracking an invasive honey bee pest: mitochondrial DNA variation in North American small hive beetles. *Apidologie* **34**, 103–109. (doi:10.1051/apido:2003004)
- FAOSTAT data 2005 Data available at <http://faostat.fao.org>; Agricultural data/Agricultural production/Crops primary. Last accessed in July 2006.
- Free, J. B. 1993 *Insect pollination of crops*. London, UK: Academic Press.
- Ghazoul, J. 2005 Buzziness as usual? Questioning the global pollination crisis. *Trends Ecol. Evol.* **20**, 367–373. (doi:10.1016/j.tree.2005.04.026)
- Ghazoul, J. 2006 Floral diversity and the facilitation of pollination. *J. Ecol.* **94**, 295–304.
- Goulson, D. 2003 Conserving wild bees for crop pollination. *Food Agric. Environ.* **1**, 142–144.
- Greenleaf, S. A. & Kremen, C. 2006 Wild bees enhance honey bees' pollination of hybrid sunflower. *Proc. Natl Acad. Sci. USA* **103**, 13 890–13 895. (doi:10.1073/pnas.0600929103)
- Greenleaf, S. A. & Kremen, C. In press. Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. *Biol. Conserv.* (doi:10.1016/j.biocon.2006.05.025).
- Heard, T. A. 1994 Behaviour and pollinator efficiency of stingless bees and honey bees on macadamia flowers. *J. Apicult. Res.* **33**, 191–198.
- Heard, T. A. & Exley, E. 1994 Diversity, abundance and distribution of insect visitors to macadamia flowers. *Environ. Entomol.* **23**, 91–100.
- Herrera, C. M., Jordano, P., Guitián, J. & Traverset, A. 1998 Annual variability in seed production by woody plants and the masting concept: reassessment of principles and relationship to pollination and seed dispersal. *Am. Nat.* **152**, 576–594. (doi:10.1086/286191)
- Higes, M., Martín, R. & Meana, A. 2006 *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *J. Inv. Pathol.* **92**, 93–95. (doi:10.1016/j.jip.2006.02.005)
- Hines, H. M. & Hendrix, S. D. 2005 Bumble bee (Hymenoptera: Apidae) diversity and abundance in tallgrass prairie patches: effects of local and landscape floral resources. *Environ. Entomol.* **34**, 1477–1484.
- Ingram, M., Nabhan, G. C. & Buchmann, S. L. 1996 Impending pollination crisis threatens biodiversity and agriculture. *Tropinet* **7**, 1.
- Javorek, S. K., Mackenzie, K. E. & Vander Kloet, S. P. 2002 Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) at lowbush blueberry (Ericaceae: *Vaccinium angustifolium* Ait.). *Ann. Entomol. Soc. Am.* **95**, 245–351. (doi:10.1603/0013-8746(2002)095[0345:CPEA BH]2.0.CO;2)
- Kearns, C. A., Inouye, D. W. & Waser, N. 1998 Endangered mutualisms: the conservation of plant–pollinator interactions. *Annu. Rev. Ecol. Syst.* **29**, 83–112. (doi:10.1146/annurev.ecolsys.29.1.83)
- Kevan, P. G. 1975 Forest application of the insecticide Fenitrothion and its effect on wild bee pollinators (Hymenoptera: Apoidea) of lowbush blueberries (*Vaccinium* spp.) in Southern New Brunswick, Canada. *Biol. Conserv.* **7**, 301–309. (doi:10.1016/0006-3207(75)90045-2)
- Kevan, P. G. & Phillips, T. 2001 The economics of pollinator declines: assessing the consequences. *Conserv. Ecol.* **5**, 8. (url:<http://www.consecol.org/vol5/iss1/art8>)
- Kevan, P. G., Clark, E. A. & Thomas, V. G. 1990 Insect pollinators and sustainable agriculture. *Am. J. Altern. Agr.* **5**, 12–22.
- Klein, A. M., Steffan-Dewenter, I. & Tscharntke, T. 2003a Fruit set of highland coffee increases with the diversity of pollinating bees. *Proc. R. Soc. B* **270**, 955–961. (doi:10.1098/rspb.2002.2306)
- Klein, A. M., Steffan-Dewenter, I. & Tscharntke, T. 2003b Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *J. Appl. Ecol.* **40**, 837–845. (doi:10.1046/j.1365-2664.2003.00847.x)
- Knight, T. M., Steets, J. A. & Ashman, T.-L. 2006 A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *Am. J. Bot.* **93**, 271–277.
- Kremen, C. & Chaplin, R. In press. Insects as providers of ecosystem services: crop pollination and pest control. In *Insect conservation biology. Proceedings of the Royal Entomological Society's 23rd Symp.* (ed. A. J. A. Stewart, T. R. New, & O. T. Lewis), Wallingford, UK: CABI Publishing.
- Kremen, C., Williams, N. M. & Thorp, R. W. 2002 Crop pollination from native bees at risk from agricultural intensification. *Proc. Natl Acad. Sci. USA* **99**, 16 812–16 816. (doi:10.1073/pnas.262413599)
- Kremen, C., Williams, N. M., Bugg, R. L., Fay, J. P. & Thorp, R. W. 2004 The area requirements on an ecosystem service: crop pollination by native bee communities in California. *Ecol. Lett.* **7**, 1109–1119. (doi:10.1111/j.1461-0248.2004.00662.x)
- Larsen, T. H., Williams, N. & Kremen, C. 2005 Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecol. Lett.* **8**, 538–547. (doi:10.1111/j.1461-0248.2005.00749.x)
- Larson, B. M. H. & Barrett, S. C. H. 2000 A comparative analysis of pollen limitation in flowering plants. *Biol. J. Linn. Soc.* **69**, 503–520. (doi:10.1006/bijl.1999.0372)
- Matheson, A., Buchmann, S. L., O'Toole, C., Westrich, P. & Williams, J. H. 1996 *The conservation of bees*. London, UK: Academic Press.
- McGregor, S. E. 1976 Insect pollination of cultivated crop-plants. *U.S.D.A. Agriculture Handbook No. 496*, 93–98. Version with some updated information for some crop species available at <http://gears.tucson.ars.ag.gov/book/>.
- Morandin, L. A. & Winston, M. L. 2005 Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecol. Appl.* **15**, 871–881.
- Morandin, L. A. & Winston, M. L. 2006 Pollinators provide economic incentive to preserve natural land in agroecosystems. *Agric. Ecosyst. Environ.* **116**, 289–292. (doi:10.1016/j.agee.2006.02.012)
- Morse, R. & Calderone, N. W. 2000 The value of honey bees as pollinators of U.S. Crops in 2000. *Bee Culture* **128**, 1–15.
- Nabhan, G. P. & Buchmann, S. 1997 Services provided by pollinators. In *Nature's services: societal dependence on natural ecosystems* (ed. G. G. Daily), pp. 133–150. Washington, DC: Island Press.
- Ortega, E., Egea, J., Cánovas, J. A. & Dicenta, F. 2002 Pollen tube dynamics following half- and fully-compatible

- pollinations in self-compatible almond cultivars. *Sex. Plant Reprod.* **15**, 47–51. (doi:10.1007/s00497-002-0137-5)
- O'Toole, C. 1993 Diversity of native bees and agroecosystems. In *Hymenoptera and biodiversity* (ed. J. La Salle & I. D. Gould), pp. 169–196. London, UK: CAB International.
- Palmer, M. et al. 2004 Ecology for a crowded planet. *Science* **304**, 1251–1252. (doi:10.1126/science.1095780)
- Parker, F. D., Batra, S. W. T. & Tependino, V. J. 1987 New pollinators for our crops. *Agri. Zool. Rev.* **2**, 279–304.
- Pías, B. & Guitián, P. 2006 Breeding system and pollen limitation in the masting tree *Sorbus aucuparia* L. (Rosaceae) in the NW Iberian Peninsula. *Acta Oecol.* **29**, 97–103. (doi:10.1016/j.actao.2005.08.005)
- Potts, S. G., Vulliamy, B., Robert, S., O'Toole, C., Dafni, A., Neeman, G. & Willmer, P. 2005 Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecol. Entomol.* **30**, 78–85. (doi:10.1111/j.0307-6946.2005.00662.x)
- Prescott-Allen, R. & Prescott-Allen, C. 1990 How many plants feed the world? *Conserv. Biol.* **4**, 366–374. (doi:10.1111/j.1523-1739.1990.tb00310.x)
- Pywell, R. F., Warman, E. A., Hulmes, L., Hulmes, S., Nuttall, P., Sparks, T. H., Critchley, C. N. R. & Sherwood, A. 2006 Effectiveness of new agri-environment schemes in providing foraging resources for bumblebees in intensively farmed landscapes. *Biol. Conserv.* **129**, 192–206. (doi:10.1016/j.biocon.2005.10.034)
- Rathcke, B. J. & Jules, E. 1994 Habitat fragmentation and plant/pollinator interactions. *Curr. Sci.* **65**, 273–278.
- Richards, K. W. 1996 Comparative efficacy of bee species for pollination of legume seed crops. In *The conservation of bees* (ed. A. Matheson, S. L. Buchmann, C. O'Toole, P. Westrich & J. H. Williams), pp. 81–103. London, UK: Academic Press.
- Richards, A. J. 2001 Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? *Ann. Bot.* **88**, 165–172. (doi:10.1006/anbo.2001.1463)
- Ricketts, T. 2004 Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conserv. Biol.* **18**, 1262–1271. (doi:10.1111/j.1523-1739.2004.00227.x)
- Ricketts, T., Daily, G. C., Ehrlich, P. R. & Michener, C. D. 2004 Economic value of tropical forest to coffee production. *Proc. Natl Acad. Sci. USA* **101**, 12 579–12 582. (doi:10.1073/pnas.0405147101)
- Robinson, W. S., Nowogrodzki, R. & Morse, R. A. 1989a Pollination parameters. *Glean. Bee Cult.* **117**, 148–152.
- Robinson, W. S., Nowogrodzki, R. & Morse, R. A. 1989b The value of honey bees as pollinators of U.S. crops. *Am. Bee J.* **129**, 411–423 see also pp. 477–478
- Roubik, D. W. 1995 Pollination of cultivated plants in the tropics. Food and agriculture organization of the United Nations, Rome, Italy. Bull. 118.
- Roubik, D. W. 2001 Ups and downs in pollinator populations: when is there a decline? *Conserv. Ecol.* **5**, 2. (<http://www.consecol.org/vol5/iss1/art2/>)
- Roubik, D. W. 2002 The value of bees to the coffee harvest. *Nature* **417**, 708. (doi:10.1038/417708a)
- Shuler, R. E., Roulston, T. H. & Farris, G. E. 2005 Farming practices influence wild pollinator populations on squash and pumpkin. *J. Econ. Entomol.* **98**, 790–795.
- Slaa, E. J., Sánchez, C. L. A., Malagodi-Braga, K. S. & Hofstede, F. E. 2006 Stingless bees in applied pollination. Practice and perspectives. *Apidologie* **37**, 293–315. (doi:10.1051/apido:2006022)
- Southwick, E. E. & Southwick Jr, L. 1992 Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *J. Econ. Entomol.* **85**, 621–633.
- Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C. & Tscharntke, T. 2002 Scale-dependent effects of landscape structure on three pollinator guilds. *Ecology* **83**, 1421–1432.
- Steffan-Dewenter, I., Potts, S. G. & Packer, L. 2005 Pollinator diversity and crop pollination services are at risk. *Trends Ecol. Evol.* **20**, 651–652. (doi:10.1016/j.tree.2005.09.004)
- Steffan-Dewenter, I., Klein, A. M., Alfert, T., Gaebele, V. & Tscharntke, T. 2006 Bee diversity and plant-pollinator interactions in fragmented landscapes. In *Specialization and generalization in plant-pollinator interactions* (ed. N. M. Waser & J. Ollerton), pp. 387–408. Chicago, IL: Chicago Press.
- Stewart, A. M. 1989 Factors affecting pollinator effectiveness in *Feijoa sellowiana*. *New Zeal. J. Crop Hort.* **17**, 145–154.
- Sundriyal, M. & Sundriyal, R. C. 2004 Wild edible plants of the Sikkim Himalaya: nutritive values of selected species. *Econ. Bot.* **58**, 286–299. (doi:10.1663/0013-0001(2004)058[0286:WEPOTS]2.0.CO;2)
- Torchio, P. F. 1990 Diversification of pollination strategies for U.S. crops. *Environ. Entomol.* **19**, 1649–1656.
- Tscharntke, T., Klein, A. M., Kruess, A., Steffan-Dewenter, I. & Thies, C. 2005 Landscape perspectives on agricultural intensification and biodiversity-ecosystem service management. *Ecol. Lett.* **8**, 857–874. (doi:10.1111/j.1461-0248.2005.00782.x)
- Tylianakis, J. M., Klein, A. M. & Tscharntke, T. 2005 Spatiotemporal variation in the diversity of Hymenoptera across a tropical habitat gradient. *Ecology* **86**, 3296–3302.
- Veddeler, D., Klein, A. M. & Tscharntke, T. 2006 Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos* **112**, 594–601. (doi:10.1111/j.0030-1299.2006.14111.x)
- Watanabe, M. E. 1994 Pollination worries rise as honey bees decline. *Science* **265**, 1170.
- Westerkamp, C. & Gottsberger, G. 2000 Diversity pays in crop pollination. *Crop Sci.* **40**, 1209–1222.
- Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. 2003 Mass-flowering crops enhance pollinator densities at a landscape scale. *Ecol. Lett.* **6**, 961–965. (doi:10.1046/j.1461-0248.2003.00523.x)
- Westrich, P. 1996 Habitat requirements of central European bees and the problems of partial habitats. In *The conservation of bees* (ed. A. Matheson, S. L. Buchmann, C. O'Toole, P. Westrich & H. Williams), pp. 1–16. London, UK: Linnean Society of London and the International Bee Research Association by Academic Press.
- Williams, I. H. 1994 The dependences of crop production within the European Union on pollination by honey bees. *Agri. Zool. Rev.* **6**, 229–257.
- Williams, I. H. 1996 Aspects of bee diversity and crop pollination in the European Union. In *The conservation of bees* (ed. A. Matheson, S. L. Buchmann, C. O'Toole, P. Westrich & H. Williams), pp. 63–80. London, UK: Linnean Society of London and the International Bee Research Association by Academic Press.
- Williams, I. H., Corbet, S. A. & Osborne, J. L. 1991 Beekeeping, wild bees and pollination in the European community. *Bee World* **72**, 170–180.
- Williams, N. M., Minckley, R. L. & Silveira, F. A. 2001 Variation in native bee faunas and its implications for detecting community changes. *Conserv. Ecol.* **5**, 7.
- Winfree, R., Griswold, T. & Kremen, C. In press. A positive response from bee pollinators to human disturbance in a forested ecosystem. *Conserv. Biol.*
- Wood, G. W. 1979 Recuperation of native bee populations in blueberry fields exposed to drift of fenitrothion from forest spray operations in New Brunswick. *J. Econ. Entomol.* **72**, 36–39.

**TAB 2**



3. In 1997, I purchased California Minnesota Honey Farms from my father-in-law. California Minnesota Honey Farms is a migratory beekeeping operation based in Eagle Bend, Minnesota and Oakdale, California. In addition to myself, California Minnesota Honey Farms employs one full time employee, as well as three seasonal employees. I maintain residences in both Eagle Bend, Minnesota and Oakdale, California.

4. For as long as I have been associated with California Minnesota Honey Farms, we have followed essentially the same annual schedule. Each fall, we load our hives onto tractor trailers in Minnesota and travel to California. There, I contract with almond, cherry, and blueberry growers in the central San Joaquin Valley to provide pollination services during the peak bloom period, which generally lasts from early February through March. After a short amount of time then spent in the Sierra foothills, I return to Minnesota in May and disperse my hives around the Eagle Bend area. Eagle Bend is in the central part of the state which is primarily agricultural of mixed type. I harvest honey in the late summer and early fall, and prepare for the trip back to California.

5. In the past, I would return to Minnesota each spring with about 3,000 bee hives. I used to expect to lose 16% of those hives over the course of the summer and the following winter. I was able to recover these annual losses by

dividing about 25% of the healthy colonies to maintain a roughly consistent colony count.

6. Since about 2004-05, the percentage of hives lost each year has increased dramatically. In 2012, for example, I had 3,150 hives in April, but by February 2013, I was down to just 998 hives, meaning I lost almost 70% of my hives just in the last year. Attempting to replace this loss, I fed the bees feed supplements and, after getting the hives back into an improved condition, split about 130% of the colonies (meaning that for the larger colonies I split them more than once). In addition, I traded with three other bee operations for an additional 300 hives. Even after all these beekeeping heroics, I was only able to bring my spring count to 2750 hives, 250 hives less than what I considered a healthy, normal level for spring in my many years of operation prior to 2005.

7. Not only am I losing hives at rates that are unprecedented, but remaining live hives are far less robust. It is plain from this year and recent years that I am getting significant *summer* mortality – a time when bee populations should be healthy due to warm weather, long days, and food abundance – from the dominant Minnesota crops, corn and soybeans. It is impossible for me to avoid soybeans in central Minnesota.

8. In Minnesota nearly all the agricultural crops are now seed-treated with a combination of two neonicotinoid pesticides, clothianidin and

thiamethoxam, and three widely-used fungicides. These seed treatments are generally considered “systemic” pesticides, a portion of which are intended to be taken up by the plant, making the plant toxic to pests. Other pesticides might be applied to the leaves, stems, or blooms of a plant to kill or repel pests. Sulfoxaflor is a pesticide that works like other neonicotinoids on pests and is registered for “foliar” application in Minnesota on soybeans and barley. That means my bees will now be exposed to sulfoxaflor just about anywhere I place them in Minnesota.

9. I anticipate summer problems from sulfoxaflor as exposures to contaminated nectar and pollen on flowering soybeans can disrupt the queens by causing a shut down in the brood rearing cycle just when the hives should be raising healthy bees for overwintering. Not only do queens shut down laying, but queen superseding can occur – this is where a new queen takes over for an old queen – an event that normally only happens once every two to three years but now is happening in my hives multiple times a year. There is no way to requeen hives at this time of year and make a viable hive for overwintering which also means less hives for spring pollination. All of the summer exposure leads to hives going into fall with substandard clusters with reduced numbers of winter-ready bees needed for meeting my pollination contract obligations in California.

10. There are several other consequences of this hive health pattern which adversely affect my business and livelihood. First is that sick or poorly-populated

hives cannot produce as much honey. This is very apparent when observing my annual honey production records. Prior to 2005, I would expect to harvest an average 80 pounds of honey per live hive annually, primarily over the course of the summer and fall. In recent seasons, I have averaged only about 58 pounds of honey per hive. This year it appears that my production has dropped again and will be in the low-30 pound per hive range. Whereas honey production used to account for about 75% of California Minnesota Honey Farm's yearly income and pollination services accounting for the remaining 25%, today the near reverse is true: honey production now accounts for only about 25% of our yearly income. What this means in dollars and cents for honey production at today's bulk price of \$2.20/lb is a honey check of about \$135,300 instead of what it should be \$435,600.

11. Since 2005, the number of hives available for pollination contracts has dropped sharply. Simple supply and demand means that fulfilling pollination obligations has become more difficult and more costly for the almond and fruit growers that rely on those contracts.

12. I take every live hive to California with the hope of using it for paid pollination services in almonds, cherries, apples, and blueberries. These specialty crop growers depend on me to bring viable hives in adequate numbers to pollinate their crops. Because of the high mortality rate, I have been forced to reduce the number of pollination contracts I sign (so while the price for pollination contracts

has risen some, I can no longer fulfill as many contracts). Because I am very well connected in the bee industry, until last spring I was able to subcontract with other beekeepers to cover the shortfall caused by pesticide exposures and losses of hives during preceding summers in Minnesota. For the 2013 pollination season, I was not able to cover the shortfall because of such extensive losses throughout the industry. In fact, there were shortfalls across the industry. I am aware of a number of growers that ended up renting substandard hives and/or simply came up short in the number of hives they wished to rent for the 2013 season.

13. Based on 2012 pricing and historic mortality, I should have rented 2562 hives of the 3050 I started with at \$210 each or \$538,020. Instead, I pulled most of my hives out of cherry pollination in order to do supplemental feeding of the hives to prepare them for splitting. I rented only 998 hives into almonds and 80 hives into cherries for a combined gross income of \$163,800.

14. When considering the combination of both honey and pollination for the 2012 season, I should have grossed \$1,511,640 instead of the measly \$299,100 I actually grossed. I cannot speak to the amount of revenue lost by the crops that were not pollinated or inadequately pollinated in the last two seasons, but given the shortage of available hives – and that I know some growers went without – I can only conclude it had an effect.

15. The U.S. Environmental Protection Agency (“EPA”) is fully aware of the situation that I have described above. I personally have been involved in touring with EPA representatives once in 2012 and twice in 2013 during almond pollination, pointing out to them the shortage of hives and the impact on beekeepers and growers. In 2012, members of the group that I toured with included Senior Scientist Tom Steeger from EPA, and in 2013 members of the group included Anita Pease, Don Brady, Jim Jones, all from EPA. In these tours, the issues with summer pesticide exposures and the tie-in with current beehive health was thoroughly discussed. I specifically raised and argued that exposure to neonicotinoid pesticides is a proximate cause of my excessive beehive mortality and that registration of sulfoxaflor will simply make that situation much worse as my bees will be exposed to highly bee-toxic pesticides everywhere, all of the time.

16. I am not the only commercial beekeeper who is suffering. My professional colleagues in both Minnesota and California as well as beekeepers in other states who I know through my membership in trade groups report similar stories of unprecedented bee die-offs since the mid-2000s. Unless current trends are reversed, there is a genuine sense that commercial beekeeping will no longer be an economically viable enterprise. I already know of bee-keepers that have left the profession due to economic losses of this nature.

17. As part of keeping up with the latest information in my profession and my role in various beekeeping organizations, I educate myself and keep abreast of reports and developments regarding beekeeping economics, practices, and bee health by reading various trade, scientific, and government publications. Based upon that information, and based upon my almost 40 years of experience as a profession beekeeper, it is my professional opinion that the current crises facing honeybees can be attributed in large part to exposure to systemic pesticides like sulfoxaflor. EPA's own analysis finds that sulfoxaflor is extremely highly toxic to honeybees, systemic, and environmentally persistent, yet the agency approved registration on numerous blooming crops with no effective labeling mitigation measures that have been demonstrated to protect bees.

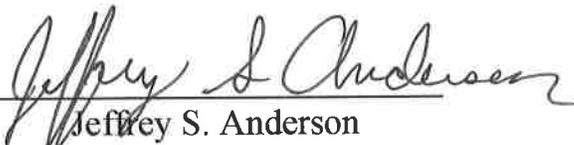
18. There are thousands of acres of soybeans, a crop for which sulfoxaflor is registered in Minnesota, within easy reach of my foraging bees. Bees can forage up to 5 miles from the hives depending on forage quality and availability. Any crop map for Minnesota will show that soybeans are one of two almost-exclusive crops in the central and southern part of the state. (The other is corn, which is also heavily treated with a neonicotinoid pesticides, providing no refuge anywhere.)

19. EPA's decision to register yet another pesticide that is highly-toxic to bees – sulfoxaflor – injures me directly. I am concerned that sulfoxaflor could be the final nail in the coffin for my business. I am participating in this action to

require EPA to reconsider its registration of sulfoxaflor, taking into account not just acute, but also chronic and sub-lethal impacts to the entire colony, and to consider those impacts as part of a cumulative problem with pesticides and bees over the last ten years. I also believe that in reconsidering the registration, EPA must take into account the very real and very negative impacts these pesticides have had and will have to the bee and food industries. Requiring EPA to reconsider the registration of sulfoxaflor and take these things into account will hopefully begin to reverse the damage from pesticide registrations that have not properly considered these issues over the last ten years.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge and belief.

Dated: October 14, 2013

  
Jeffrey S. Anderson

**TAB 3**

**UNITED STATES COURT OF APPEALS  
FOR THE NINTH CIRCUIT**

POLLINATOR STEWARDSHIP COUNCIL,	)	
et al.	)	Docket No. 13-72346
	)	
Petitioners,	)	
v.	)	
	)	
UNITED STATES ENVIRONMENTAL	)	
PROTECTION AGENCY, et al.	)	
	)	
Respondents,	)	
and	)	
	)	
DOW AGROSCIENCS LLC.	)	
	)	
Respondent-Intervenor.	)	
_____	)	

**DECLARATION OF GEORGE K. HANSEN**

I, George K. Hansen, declare as follows:

1. I am a professional beekeeper, and co-owner of Foothills Honey Company LLC, located in Colton, Oregon. I have personal knowledge of the matters stated herein and, if called as a witness, could and would competently testify thereto.

2. In business for more than three decades, Foothills Honey Company maintains approximately 5,000 hives and employs five full-time workers plus additional seasonal workers. Like most Pacific Northwest apiaries, Foothills

Honey Company derives the majority of its income from commercial pollination services. We contract with growers to provide pollination services for a wide variety of vegetable seed crops – including several cucurbits – in Oregon, as well as almonds in California and tree fruits in Washington.

3. I am currently serving a two-year term as the president of the American Beekeeping Federation (“ABF”). I was the vice president of ABF for two years prior to becoming president, and prior to that I was a member of ABF’s board of directors. As a professional beekeeper, I have been a member of ABF since 1993.

4. ABF is a 501(c)(4) organization founded in 1943, headquartered in Atlanta, Georgia. ABF is dedicated to advancing the interests of all beekeepers, large or small, and other interests associated with the industry to ensure the future of the honey bee. ABF currently has approximately 1,300 members, making it the largest beekeeping organization in the United States. I estimate that approximately 25% of the commercial beekeepers in the United States are members of ABF. Collectively, I estimate that ABF members harvest roughly 30% of the honey produced in the United States each year, a lot of that from smaller producers.

5. In my capacity as president of ABF, I travel frequently to beekeeping conferences and other events at which I discuss issues facing the beekeeping industry with ABF members and other beekeepers. I also meet frequently with

state and federal government officials, including officials and staff at the U.S. Environmental Protection Agency (“EPA”) and U.S. Department of Agriculture (“USDA”), to discuss issues facing honeybees and beekeepers. I also keep abreast of publications, whether from the industry, government, or the science community, that are germane to economic or industry practice issues or issues concerning bee health.

6. Beginning around the mid-2000s, ABF members began reporting a marked increase in the percentage of colonies lost each year. In October 2006, some of our members began reporting losses of 30 to 90 percent of their hives. While colony losses are not completely unexpected, especially over the winter, the magnitude of these losses was extraordinarily high. I understand from my reading and research as well as from my extensive work with government and industry leaders that this increase roughly coincides with the introduction and then steady increase of neonicotinoids and other similar systemic pesticides, of which sulfoxaflor is the most recent addition.

7. I have reviewed preliminary results of the 7th annual national survey of honey bee colony losses, conducted by the Bee Informed Partnership and USDA and available online at <http://beeinformed.org/2013/05/winter-loss-survey-2012-2013/>. The preliminary results indicate that 31.1% of managed honey bee colonies in the United States were lost during the 2012/2013 winter. The 6,287 beekeepers

who participated in the seventh annual survey indicated that they considered a loss rate of 15% as “acceptable,” but 70% of them suffered losses greater than this, often much higher.

8. As a commercial pollinator, brood development and colony strength are the basis of my business. Whereas Foothills Honey Company would occasionally experience catastrophic losses in some hive groups from pesticide applications early in my career, lately the damage is rarely so immediately dramatic. Rather, our losses from pesticides increasingly reflect an overall colony deterioration that takes place over time, resulting in consistently weakened bees and colonies. Also, colony failures are no longer isolated.

9. Colony decline is extremely costly. In order to keep our hive count available for pollination, we must annually rebuild and hold in reserve 35 to 50 percent of our hive count in order to take the place of hives that are failing. This costs time and labor and results in lost income. Again, this trend generally coincides with the increased use of pesticides like sulfoxaflor that cause chronic in addition to acute damage to the colonies.

10. In October 2012, scientists from USDA, EPA and other government agencies convened a conference in Alexandria, Virginia that brought together stakeholders with expertise in honey bee health. I attended the conference and have reviewed the final report that came out of that conference, which is available

online at [www.usda.gov/documents/ReportHoneyBeeHealth.pdf](http://www.usda.gov/documents/ReportHoneyBeeHealth.pdf) and Exhibit A hereto. Among other things, the report confirms at page 5 that “[h]oney bee colonies have been dying at a rate of about 30 percent per year over the past few winters, which leave virtually no cushion of bees for pollination.” The report also confirms at page vi that “effects of pesticides on honey bees have been increasingly documented, and are a primary concern.”

11. Sulfoxaflor is likely to be used on many of the crops that our bees pollinate, and it has been approved for use on a variety of crops that are in close proximity to our hives and where we are working. For example, it has been approved for use on many fruits and vegetables most of which are grown in Oregon, Washington, and California. As a result of EPA’s decision to register sulfoxaflor, my bees will be exposed to yet another pesticide that is both acutely and chronically toxic to bees, not only through drift or direct application but also through foraging on or nearby crops that have been sprayed. The reality is that if pollen is infected with a systemic pesticide like sulfoxaflor, it can be taken back to the hive. It will be impossible for me to continue my business and avoid exposure to sulfoxaflor.

12. An estimated one-third of all food and beverages are made possible by pollination, mainly by honey bees. In the United States, pollination contributes to crop production worth \$20-30 billion in agricultural production annually. A

decline in managed bee colonies puts great pressure on the sectors of agriculture reliant on commercial pollination services.

13. EPA's decision to register sulfoxaflor harms me. Had EPA obtained adequate information regarding toxicity to honeybees, I am confident that it would have either refused to register sulfoxaflor or else required substantial and adequate mitigation measures to protect pollinators. By contrast, EPA's suggestion that applicators avoid spraying between 7 a.m. and 7 p.m. is not protective and not supported by the actual facts of bee foraging. Putting aside the fact that the advisory is not enforceable, the only time bees are not foraging in Oregon is in full dark. Similarly, extending the time between applications does little to address the chronic or sub-lethal impacts of pesticides like sulfoxaflor that are affecting our colonies. By requiring EPA to go back and reanalyze all of the impacts of sulfoxaflor to the bee industry – including the impact of chronic exposure on colonies and the economic impacts of the pollinator industry on agriculture and the food supply – I expect that EPA will reach a better-considered decision that is more fully informed regarding the registration and/or use of sulfoxaflor.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge and belief.

Dated: October 27, 2013

  
George K. Hansen, President,  
American Beekeeping Federation

# Exhibit A

To Declaration of George K. Hansen

# **Report on the National Stakeholders Conference on Honey Bee Health**

**National Honey Bee Health Stakeholder Conference Steering Committee**

A close-up photograph of two honey bees on a bright yellow flower. The bees are positioned on the green, textured center of the flower, surrounded by vibrant yellow petals. The background is softly blurred, showing more of the flower and green leaves.

**Sheraton Suites Old Town Alexandria Hotel  
Alexandria, Virginia  
October 15–17, 2012**

**National Honey Bee Health Stakeholder Conference Steering Committee**

USDA Office of Pest Management Policy (OPMP)

David Epstein

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James L. Frazier

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USDA Natural Resources Conservation Service (NRCS)

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U. S. Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP)

Thomas Moriarty

Thomas Steeger

**Disclaimer:**

**This is a report presenting the proceedings of a stakeholder conference organized and conducted by members of the National Honey Bee Health Stakeholder Conference Steering Committee on October 15-17, 2012 in Alexandria, VA. The views expressed in this report are those of the presenters and participants and do not necessarily represent the policies or positions of the Department of Agriculture (USDA), the Environmental Protection Agency (EPA), or the United States Government (USG).**

## **Executive Summary**

After news broke in November 2006 about Colony Collapse Disorder (CCD), a potentially new phenomenon described by sudden and widespread disappearances of adult honey bees from beehives in the U.S., the CCD Steering Committee was formed with the charge to help coordinate a federal response to address this problem. The CCD Steering Committee consists of scientists from the Department of Agriculture's (USDA) Agricultural Research Service (ARS), National Institute of Food and Agriculture (NIFA), Animal Plant Health Inspection Service (APHIS), Natural Resources Conservation Service (NRCS), Office of Pest Management Policy (OPMP), the National Agricultural Statistics Service (NASS), and also includes scientists from the Environmental Protection Agency (EPA). At that time, the Committee requested input and recommendations from a broad range of experts in apiculture about how to approach the problem. Out of this, the steering committee developed the CCD Action Plan ([www.ars.usda.gov/is/br/ccd/ccd\\_actionplan.pdf](http://www.ars.usda.gov/is/br/ccd/ccd_actionplan.pdf)), which outlined the main priorities for research and outreach to be conducted to characterize CCD and to develop measures to mitigate the problem. Since formation of the CCD Steering Committee early in 2007, the USDA, EPA and public and private partners have invested considerable resources to better address CCD and other major factors adversely affecting bee health.

Despite a remarkably intensive level of research effort towards understanding causes of managed honeybee colony losses in the United States, overall losses continue to be high and pose a serious threat to meeting the pollination service demands for several commercial crops. Best Management Practice (BMP) guides have been developed for multiple stakeholders, but there are numerous obstacles to widespread adoption of these practices. In addition, the needs of growers and other stakeholders must be taken into consideration before many practices can be implemented.

To address these needs, several individuals from the CCD Steering Committee, along with Pennsylvania State University, organized and convened a conference on October 15-17, 2012, in Alexandria, Virginia that brought together stakeholders with expertise in

honey bee health. Approximately 175 individuals participated, including beekeepers, scientists from industry/academia/government, representatives of conservation groups, beekeeping supply manufacturers, commodity groups, pesticide manufacturers, and government representatives from the U.S., Canada, and Europe.

A primary goal of the conference was for the CCD Steering Committee to receive input from stakeholders as they consider future actions to promote health and mitigate risks to managed honey bees in the United States. The meeting had three objectives:

1) Synthesize the current state of knowledge regarding CCD, bee pests, pathogens, and nutrition, potential pesticide effects on bees, and bee biology, genetics and breeding; 2) Facilitate the development and implementation of BMPs that stakeholders can realistically incorporate; and 3) Identify priority topics for research, education and outreach to be considered by the CCD Steering Committee for an updated Action Plan.

Dr. May Berenbaum gave the keynote address and provided an overview of the historical and current state of pollinators in the United States, from the invention of the first movable hive frame in 1852 and the first printed reference to non-target impacts of agricultural pesticides on bees in 1891, through the first U.S. detection of the parasitic *Varroa* mite in 1987 and the more recent colony declines over the past decade. Leaders in apicultural research gave comprehensive presentations of research progress on CCD, bee pests and pathogens, nutrition, pesticides, bee biology, breeding and genetics.

**Highlights of Research Overviews:** *As noted earlier, the views expressed in this report are those of the presenters and do not necessarily represent the policies or positions of the U.S. Department of Agriculture, the Environmental Protection Agency, or the United States Government.*

- Consensus is building that a complex set of stressors and pathogens is associated with CCD, and researchers are increasingly using multi-factorial approaches to studying causes of colony losses.

- The parasitic mite *Varroa destructor* remains the single most detrimental pest of honey bees, and is closely associated with overwintering colony declines.
- Multiple virus species have been associated with CCD.
- Varroa is known to cause amplified levels of viruses.
- The bacterial disease European foulbrood is being detected more often in the U.S. and may be linked to colony loss.
- Nutrition has a major impact on individual bee and colony longevity.
- Research indicates that gut microbes associated with honey bees play key roles in enhancement of nutrition, detoxification of chemicals, and protection against diseases.
- Acute and sublethal effects of pesticides on honey bees have been increasingly documented, and are a primary concern. Further tier 2 (semi-field conditions) and tier 3 (field conditions) research is required to establish the risks associated with pesticide exposure to U.S. honey bee declines in general.
- The most pressing pesticide research questions lie in determining the actual field-relevant pesticide exposure bees receive and the effects of pervasive exposure to multiple pesticides on bee health and productivity of whole honey bee colonies.
- Long-term cryopreservation of honey bee semen has been successfully developed and provides the means for long-term preservation of “top-tier” domestic honey bee germplasm for breeding. Genetic variation improves bee thermoregulation, disease resistance and worker productivity.
- Genomic insights from sequencing the honey bee genome are now widely used to understand and address major questions of breeding, parasite interactions, novel controls (*e.g.*, RNAi), and management to make bees less stressed and more productive.

To facilitate discussion of BMPs and development of priorities, stakeholders were formed into work groups centered on the four main issues affecting bee health: 1) nutrition, 2) pesticides, 3) parasites/pathogens and 4) genetics/ biology/ breeding. The most common themes expressed in several breakout groups were:

- Federal and state partners should consider actions affecting land management to maximize available nutritional forage to promote and enhance good bee health and to protect bees by mitigating their movement into pesticide-treated crop acreage.
- Undernourished or malnourished bees appear to be more susceptible to pathogens, parasites, and other stressors, including toxins. Research is needed on forage, pollen, insect metabolic pathways, artificial and natural food sources, and food processing and storage in the hive.
- More outreach programs targeting farmers on managing potential exposure of honey bees to pesticides is needed. Efforts would benefit from involvement of beekeepers, crop consultants, pesticide manufacturers and applicators, and State lead agencies and extension agents.
- BMPs associated with bees and pesticide use, exist, but are not widely or systematically implemented by members of the crop producing industry. A central theme of the pesticides session was the need for informed and coordinated communication/education/extension of growers and beekeepers and the need for effective collaboration between stakeholders.
- Beekeepers accentuated the need for accurate and timely beekill incident reporting, monitoring, and enforcement.
- Pathogens and arthropod pests have major negative impacts on colonies. Management of *Varroa* and viruses was recognized as a special concern.
- Breeding emphasis is on traits, including hygienic behavior, that confer improved resistance to *Varroa* mites and diseases, such as American Foulbrood.

Although a post meeting survey was not conducted, meeting participants indicated that the conference gave them the opportunity to voice their concerns, to hear the concerns of others, and to offer their perspectives to Federal officials on future directions the government might take to ensure the future of America's pollinators. The CCD Steering Committee plans to revise the CCD Action Plan, a document that will synthesize this input. The Action Plan will outline major priorities to be addressed in the next 5-10 years. This plan will serve as a reference document for policy makers, legislators and the public and to help coordinate the federal strategy in response to honey bee losses. Finally,

given the depth of issues effecting pollinator health, consideration should be given to renaming this committee to reflect the broader range of factors discussed in this report.

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## Background

In response to unexplained losses of U.S. honey bee (*Apis mellifera*) colonies that began to be reported in 2006 as a condition named Colony Collapse Disorder (CCD), the U.S. Department of Agriculture (USDA) established a Colony Collapse Steering Committee to lead an effort to define an approach for understanding and resolving the problem. CCD is characterized by the sudden loss of worker adults from managed hives, leading to the eventual collapse of the entire colony within a few weeks. It is a complex phenomenon, because several factors seem to be interacting to cause CCD (<http://www.ars.usda.gov/is/br/ccd/ccdprogressreport2012.pdf>) (CCD Progress Report 2012). The suspected factors include pests, pathogens, pesticides, nutritional deficiencies and bee hive management practices. The CCD Steering Committee, formally established in 2007, was initially composed of program leaders from ARS, NIFA, APHIS, NRCS, and NASS, the U.S. Environmental Protection Agency Office of Pesticide Programs (EPA) and two land-grant university administrators from Pennsylvania State University and Purdue University. Using input and recommendations received by university and government researchers, extension specialists and beekeepers, the steering committee developed the CCD Action Plan in July 2007 to establish key priorities for research and development of management practices to address CCD ([http://www.ars.usda.gov/is/br/ccd/ccd\\_actionplan.pdf](http://www.ars.usda.gov/is/br/ccd/ccd_actionplan.pdf)). Currently, the steering committee includes USDA's Office of Pest Management Policy (OPMP); formal participation of the two land-grant universities ended.

In the past five years, significant progress has been made in our understanding of the factors that are associated with CCD and the overall health of honey bees. Survey data generated by USDA (<http://www.ars.usda.gov/is/pr/2011/110523.htm>) indicate that overwinter losses for commercial beekeepers ranged from approximately 28 to 33 percent between 2007 and 2011 and were reported as 22 percent in 2012. It was noted in 2010-11 winter loss survey that fewer beekeepers attributed losses to CCD than in previous years (vanEngelsdorp et al. 2012), even though those reporting CCD as the cause of their losses suffered higher than average losses. Nevertheless, overall losses far exceed the historical rate (approximately 10 to 15 percent) and represent a threat to both beekeepers and to those agricultural crops that rely upon pollination as a production input. Since 2006 an estimated 10 million bee hives at an approximate current value

of \$200 each have been lost and the total replacement cost of \$2 billion dollars has been borne by the beekeepers alone (J. Frazier, unpublished).

Members of the CCD Steering Committee believed that, after five years of investigating CCD, it was necessary to assess the current state of knowledge of CCD, and of the primary factors that affect honey bee health. To this end, a subcommittee formed to plan and conduct a stakeholder conference, with the objective of seeking input from the stakeholder community regarding current understanding of research priorities, and the development of BMP's to address the needs of beekeepers and growers.

A stakeholder conference was held on October 15-17, 2012 in Alexandria, Virginia. Approximately 150 individuals were invited to the conference, including beekeepers, scientists, representatives of advocacy groups, beekeeping supply manufacturers, commodity groups, pesticide producers, academia, and State and Federal government representatives from the U.S., Canada, and Europe. The meeting was planned, organized and conducted by representatives from multiple agencies within the USDA and the U.S. EPA, along with Dr. James Frazier, Pennsylvania State University.

#### **Conference Overview:**

The goal of the conference was for officials from USDA and U.S. EPA to receive input from scientists, state governments, non-governmental organizations, industry and other stakeholders as they consider future actions to promote health and mitigate risks to North America's managed honey bees. The meeting had four aims:

- Synthesize the state of knowledge regarding CCD

Synthesize the current state of knowledge regarding each of the factors believed to be associated with declines in

- honey bee health
  - Arthropod pests and pathogens
  - Nutrition
  - Pesticides
  - Bee biology, genetics, and breeding

- Discuss and identify priority topics for research and BMPs to be considered by the CCD Steering Committee for action

The first day of the meeting was devoted to examining current and recent (past 5 years) research on each of the above four factors known to affect honey bee health. Eleven researchers from land-grant universities and the USDA Agricultural Research Service (ARS) presented research summaries addressing each health-factor topic. On the second day of the conference, participants were assigned to one of four work groups in which they were encouraged to discuss viewpoints on one of the specific areas associated with honey bee health. Work group assignments were based on participants' knowledge in the topic area. Work group discussions were led by the researchers, who presented the research summaries on day one, and were facilitated by USDA and U.S. EPA personnel. The research leads, along with conference organizers, developed a set of questions designed to guide discussion within each work group (Appendix 2).

Participants reconvened during the afternoon of the second day, when recorders from each work group summarized the key questions and recommendations developed in the morning sessions. A general discussion session followed, which ensured that participants could contribute additional ideas to work groups other than to the one to which they had been assigned.

## **Day 1: Opening Remarks and Comments**

USDA Deputy Secretary, Kathleen Merrigan, U.S. EPA Deputy Administrator, Bob Perciasepe, and USDA National Institute of Food and Agriculture (NIFA) Director Sonny Ramaswamy each provided opening remarks, addressing the importance of the issues to be discussed during the conference, and commitments by both organizations to respond to the challenges of promoting bee health while mitigating risk.

The following representatives of several stakeholder groups were also invited to provide opening comments:

- Darren Cox, Beekeeper Representative to the EPA Pesticide Program Dialogue Committee; Cox Honeyland, Logan, Utah
- Daniel Botts, Minor Crop Farmer Alliance and Florida Fruit & Vegetable Association, Maitland, Florida
- Dr. Gabrielle Ludwig, Senior Manager of Global, Technical and Regulatory Affairs, Almond Board of California, Modesto, California
- Dr. Barbara Glenn, Senior Vice President, Science and Regulatory Affairs, CropLife America, Washington, District of Columbia
- Laurie Davies Adams, Executive Director, North American Pollinator Protection Campaign, San Francisco, California
- Christi Heintz, Executive Director, Project Apis m., Tucson, Arizona

**Day 1: Research Presentations:** The keynote speaker was Dr. May Berenbaum of the University of Illinois Urbana-Champaign, who provided a comprehensive overview of honey bee declines. Berenbaum's presentation included an overview of historical focus on the conduct of honey bee research efforts, including challenges in experimental design and conduct yielding relevant results regarding colony health.

Leading scientists who study honey bees were identified and selected by the conference steering committee to present on a range of topics associated with honey bee health. Each presentation was followed by an open forum, during which conference participants were encouraged to ask

questions or provide commentary. *Comments recorded, below, in the research summaries do not represent the expressed opinions of agencies or personnel of the USDA, the US EPA, or the U.S. Government.*

### **Current State of Knowledge of CCD and its Relation to Honey Bee Health**

*(Dr. Jeff Pettis, USDA ARS, Beltsville, Maryland; Dr. Dennis vanEngelsdorp, University of Maryland, College Park, Maryland)*

**Summary of Research Presentation:** No single silver bullet will solve the problems affecting honey bees and other pollinators. Habitat enhancement, judicious and targeted pesticide use, improved colony management techniques and improved disease and pest resistant stocks of bees are collectively needed to improve the health of honey bee colonies. It is imperative that we increase honey bee survival both to make beekeeping profitable but more importantly to meet the demands of U.S. agriculture for pollination and thus ensure of food security.

- Healthy honey bee colonies are critical for meeting the demands of food production in the United States.
- Currently, the survivorship of honey bee colonies is too low for us to be confident in our ability to meet the pollination demands of U.S. agricultural crops.
- Historically, the U.S. had as many as 6 million colonies in 1947, with declines since that time to about 4 million in 1970 and 3 million in 1990. Today's colony strength is about 2.5 million.
- Pollination demands have increased in recent years such that a single crop, almonds in California, now require over 60 percent of all managed colonies.
- Honey bee colonies have been dying at a rate of about 30 percent per year over the past few winters which leave virtually no cushion of bees for pollination.
- Because of the early almond pollination requirement, a 30 percent loss of the 2.5 million colonies would leave only 1.75 million colonies to meet the 1.5 to 1.7 million colonies currently needed in almonds. This situation leaves growers in a precarious position, and Dr. Pettis stated, "We are one poor weather event or high winter bee loss away from a pollination disaster."

- Surveys of beekeepers throughout the United States have documented this 30 percent or greater loss for five consecutive years while for the most recent winter, 2011-2012, the losses were only 22 percent.

While the lower level of loss for overwintering hives in 2011-2012 was encouraging, one year does not make a trend and reports of losses in the latter part of 2012 look like we are in for another high loss winter rate. We need to improve colony survivorship, however, honey bee health issues, including CCD, have proven to be multi-faceted and difficult to solve.

- Research into CCD and poor colony health has been unable to identify a unique causative agent but consensus is building that a complex set of stressors and pathogens can result in colony losses.
- Factors that can lead to poor health include disease and arthropod pests, pesticides, poor nutrition and beekeeping practices.
- The parasitic mite *Varroa destructor* remains the single most detrimental pest of honey bees and can magnify the role of viruses in bee health.
- Pesticide exposure to pollinators continues to be an area of research and concern, particularly the systemic pesticides such as neonicotinoids. Despite concerns regarding the potential hazard that systemic pesticides may represent to honey bee colonies, when pesticides are viewed in the aggregate at the national level, the frequency and quantity of residues of pyrethroids coupled with the toxicity of these insecticides to bees could pose a 3-fold greater hazard to the colony than the systemic neonicotinoids.
- Several studies have demonstrated that sublethal neonicotinoid exposure in immature bees resulted in an increased susceptibility to the gut pathogen *Nosema*, demonstrating that complex interaction between factors are likely contributing to poor colony health.
- Nutrition has a major impact on individual and colony longevity. There is a belief among beekeepers and researchers alike that land use patterns have changed to an extent where there is less forage available for honey bee colonies. Research is beginning to look at ways to diversify the agricultural landscape to increase resource availability for pollinators.
- The use of modern weed control methods in agriculture, forestry and States' Rights of Way land management have reduced availability of weeds that once provided valuable nutrition to bees.

## **Current State of Knowledge of Bee Biology**

(Dr. Jay Evans, USDA ARS, Beltsville, Maryland)

**Summary of Research Presentation:** Research on honey bees involves several fields of biology, and advances in these fields are just now having an impact on maintaining healthy pollinator populations in the face of biotic and abiotic threats. New genetic and experimental approaches to address pollinator health are in use.

- A challenge to the research community is how do we weigh the relative importance of behavioral and physiological traits on bee health?
  - Understanding the relative importance of individual and ‘social’ traits and the trade-offs in terms of costs of maintaining these traits, will lead to better bee breeding and management (Evans and Spivak, 2010)
  - Pathogens and parasites of honey bees have been described in great detail, linking important microbes with negative (Runckel *et al.*, 2011; Cornman *et al.*, 2012) and positive (Anderson *et al.*, 2011; Engel, Martinson, and Moran, 2012) effects on bee health.
  - The genetics behind individual bee responses to viruses, bacteria, and gut parasites like *Nosema* (Siede, Meixner, and Büchler, 2012) and of how adult bees within the hive respond to signs of disease among their nestmates (Oxley, Spivak, and Oldroyd, 2010) are becoming more clear.
  - Evidence that infected honey bees may ‘suicidally’ take risks that decrease chances they will transmit disease to nestmates (Rueppell, Hayworth, and Ross, 2010) may enable more efficient breeding programs aimed at producing disease resistant bees.
- How signals shared among & between bees & their varied pests can be exploited to:
  - Control pests, *i.e.*, by understanding how *Varroa* mites, the primary pest of honey bees worldwide, perceive vulnerable bees (Calderón *et al.*, 2009) and the means by which bees perceive and remove these mites (Harris, Danka, and Villa, 2012).
  - Manipulate foraging and other colony traits by understanding how bee behavior reflects the interplay between bee proteins, developmental stage, and environmental cues. Planned research will be extended to find key traits involved with recognizing and removing pests, such as hygienic behavior.

- Raise and maintain robust queens. For example, recent work describes how the genome of developing queen and worker bees is altered during development, revealing that a large number of genomic regions are silenced in developing bees using methylation (Foret *et al.*, 2012), a way of ‘painting’ chromosomal regions into silence or activity; previously thought to be of only minor importance for insects.
- We need consistent protocols for bee research, from genetics to field experiments in order to compare data on the impacts of parasites, pathogens, nutrition and chemicals on bee health.
  - A major effort is underway to increase common practices among bee scientists and to disseminate scientific findings to the beekeeping world. The ‘Beebook’ (Williams *et al.*, 2012) is a growing compendium of research protocols and insights that will enable more consistent experiments aimed at understanding bee health and bee biology. Information from the Beebook will be joined with the key venues for dispersing honey bee information among stakeholders, regulators, and researchers, including the USDA-funded Extension.org site for bee health ([http://www.extension.org/bee\\_health](http://www.extension.org/bee_health)), the Colony Loss network (<http://www.coloss.org/>) and the newly established Bee Informed Partnership (<http://beeinformed.org>).
- What is the current consensus on biological and abiological factors that act non-additively to impact bee health, and how do we use this knowledge?
- There is a huge shift towards multi-factorial studies in all fields of bee research. Studies of bee biology and bee health have tended to focus on one factor (one genetic trait or one environmental component) and the impacts of this factor on bee health. Recent work on non-additive interactions between chemical insults to bees and parasites (*e.g.*, Alaux *et al.*, 2010; Pettis *et al.*, 2012), and on the interplay between nutrition and disease, exemplify the benefits of looking at problems of bee health from the standpoint of multiple inputs. Other examples include:
  - The impacts of bee genetics and the environment on bee foraging (Ament *et al.*, 2012; Page Jr, Fondrk, and Rueppell, 2012)

- The effects of larvae and nurse bees on the development of new queens (Linksvayer *et al.*, 2011). This will lead to richer insights into bee biology and presumably new ideas for the management and breeding of healthy bees.
- The description of the Honey Bee Genome Project “Honeybee Genome Sequencing Consortium” (2006) has become the most-cited research paper in honey bee biology. Genomic insights are now widely used to understand and address the major questions of breeding, vetting traits, parasite interactions, novel controls (RNAi), and management to make bees less stressed and more productive.
  - Scientists are using the power of genome-wide expression analysis to understand:
    - Bee responses to *Varroa* (Nazzi *et al.*, 2012).
    - Bee responses to poor nutrition (Alaux *et al.*, 2011).
- Results from CCD-driven studies have changed dogma related to:
  - The frequency with which bees are exposed to pathogens on flowers (Singh *et al.*, 2010).
  - The global nature of bee parasites and pathogens (Fries, 2010).
  - The physiological and behavioral toll of poor nutrition and exposure to chemicals (Gregorc and Ellis, 2011; Gregorc *et al.*, 2012; Henry *et al.*, 2012).
- Conclusions and Future Efforts: There remain many major knowledge gaps in bee biology, including:
  - Impacts of nutrition and food diversity on the longevity of queens and workers.
  - Importance and maintenance of the microbiome within the bee digestive tract.
  - Movement of parasites and pathogens across species and across continents.
  - Impacts of human barriers to spread of disease agents, including trade regulation and surveys.
  - The causes and sustainable exploitation of what seems to be an immense genetic diversity of traits related to both individual and colony-level disease resistance.
  - The reasons behind what seem to be inconsistent, but important, bee losses due to exposure to pesticides and other chemicals.

### **Current State of Knowledge of Nutrition and Best Management Practices**

*(Dr. Gloria DeGrandi-Hoffman, USDA ARS, Tucson, Arizona; and Dr. Nancy Moran, Yale University, New Haven, Connecticut)*

**Summary of Research Presentation:** A broad overview of recent honey bee nutrition research was presented that spanned topics from the relationship between nutrition and colony survival to the role of microbes in food processing, preservation and digestion of nutrients. Comprehensive investigation into the role of nutrition in honey bee colony health has only recently begun at the landscape, colony and molecular levels. A more in-depth understanding of the nutritional value of pollen sources and the factors affecting nutrient acquisition will provide more accurate assessments of the nutritional benefits of different pollen sources and artificial diets. We also will be able to evaluate the effects of antibiotics and pesticides on colony growth and survival from a nutritional perspective by determining their effects on nutrient acquisition and metabolism. This work will complement the need for increased bee forage and the selection of plants that would most benefit colony growth.

Specifically, the presentation included mathematical model predictions of nutrition effects on worker longevity and the repercussions on colony growth, and vulnerability to loss from parasites, such as *Varroa* mites. Information on the nutritional value of pollen and the changes in protein and amino acid concentrations after conversion of pollen to bee bread (a mixture of plant pollen, nectar, enzymes, bacteria and fungi used as food) also was provided. Recent studies have revealed new understanding about the role of nutrients and importance of beneficial microbes on honey bee health such as:

- Nutritional stress on overwintering colonies reduces the lifespan of adult workers by four days; from an average of 35 to 31 days.
- Pollen is the key protein source and bee bread is the dominant amino acid source in honey bee diets.
- Studies from several research groups reveal a distinctive set of species of gut microbes present in adult honey bees worldwide (Africa, Australia, Asia, North America, South America, Europe)
  - Eusociality enables efficient transmission of specialized bacterial communities.

- Eight distinctive microbial species make up 95 to 99 percent of gut bacteria in most bees.
- Possible roles of microbes in bee health:
  - Biosynthesis of needed nutrients
  - Enzymes for pollen digestion
  - Detoxification of compounds in diet
  - Protection against parasites or diseases (*e.g.*, infection levels by *Crithidia* parasites) depends on which strains of gut bacteria are present in the bee.
- Bacterial species colonize specific regions of the adult honey bee gut.
- Two primary bacterial microbes are present in worker bees and in highly specific gut locations :
  - *Snodgrassella alvi*
  - *Gilliamella apicola*
- Advances in genomics research are providing unprecedented opportunities to explore diversity and function of gut microbiota. Examples of studies could include the following:
  - Bacterial sampling directly from bees or from cultured bee bacteria
  - Massive sequencing of microbial genomic DNA
  - Bioinformatics using databases from model bacterial systems
  - Predicting and validating functional capabilities of bacteria at the individual and colony level.
- Within a single bee, gut bacteria encode enzymes involved in the breakdown of dietary components, transport of sugars and amino acids, and biosynthesis of nutrients (*i.e.* Some strains of *Gilliamella* encode pectate lyase enzymes for breakdown of pectin, a major component of pollen walls).
- Antibiotic resistance genes have accumulated in bee gut bacteria in the U.S. due to history of frequent use for public and agricultural treatments.
- The presentation ended with specific questions that need to be addressed in future work.
  - What is next and what else do we need to know about honey bee microbiota?
  - What is the role of microbes in bee bread and honey in the hive?
  - Do honey bee-associated bacteria help to protect against disease?
  - What is the role of microbes in making nutrients or in utilizing dietary components?

- Can we promote beneficial microbes in the colonies by beekeeping practices?
- What are effects of antibiotic use and artificial diets on the composition and functioning of bee microbiota, both within the gut and within the colony?

### **Current State of Knowledge of Pathogens and Best Management Practices**

*(Dr. Diana Cox-Foster, Pennsylvania State University, University Park, Pennsylvania; Dr. Jay Evans, USDA ARS, Beltsville, Maryland)*

**Summary of Research Presentation:** Greater information and knowledge about the normal microflora and pathogens associated with honey bees have been revealed through next generation sequencing and epidemiological studies and surveys. There is a dynamic ecology or flux in pathogens over time within a colony and among colonies. While new species have been discovered using metagenomics, and new pathologies have been described, including melanization (chemical defense against invasion of internal tissues by pathogens) of bee organs and brood pathologies such as “snotty brood”, careful experimentation is now needed to associate novel bee health concerns with specific microbes.

- Viruses:
  - *New virus species have been characterized in bees in the United States.*
  - *Multiple virus species have been associated with CCD*
  - Closely related dicistroviruses most associated with the colonies include:
    - Israeli Acute Paralysis Virus
    - Kashmir Bee Virus
    - Acute Bee Paralysis Virus
    - No detection of slow bee paralysis virus in colonies in the U.S. to date
  - Most predominant viruses in U.S. are Deformed Wing Virus and Black Queen Cell Virus
  - *Varroa* is the primary factor known to cause amplified levels of some bee viruses
  - Other factors potentially affecting virus levels include:
    - Nutrition
    - Environmental chemicals (*i.e.* pesticides and in-hive miticides)

- Other pathogens
- Age of bees.
- Several questions exist concerning viruses
  - How do viruses kill bees and the colony?
  - How can viruses impact other aspects of colony health, such as behavior (learning), chemical communication, and reproduction?
  - What impact do viruses have on the brood?
- Viral infections have also been detected in other hymenopteran pollinators and have been shown to negatively impact alfalfa leaf cutting bees and bumble bees. This raises the question whether the decline in native hymenopteran pollinators is a result of viral infections, perhaps interacting with the same stress factors affecting honey bees.
- Bacterial:
  - New information is available on variation among American foulbrood (*Paenibacillus larvae*, the most widespread and destructive of honey bee brood diseases) strains and the potential for these bacteria to interact with other gut microbes.
  - Some American Foulbrood strains have developed antibiotic resistance.
  - European foulbrood is being detected more often and may be linked to colony loss, in contrast to its rare detection in the past years.
- Fungi:
  - Chalkbrood detected more often in colonies over the past decade.
  - There are potentially other unknown fungal pathogens in bees, since characteristic symptoms are observed in some autopsies of bees from collapsing colonies.
- Microsporidia:
  - *Nosema ceranae* – widespread occurrence in U.S. colonies with some *N. apis* also present.
    - Data support a shift in prevalence in species composition during the last 50 years to favor *N. ceranae* over *N. apis*.
    - No widespread colony losses can be attributed to *N. ceranae* in the U.S.
    - Some colony losses may be associated with microsporidia since some beekeepers that treated with fumagillin (which kills microsporidia and other fungi) have

reported fewer colony losses; although research data suggest that fumagillin may actually stress bees resulting in poor colony health

- New insights into *N. ceranae* biology and its association with bees have resulted from several cage studies.
- *Nosema* genome has been sequenced.
- *Nosema* immunosuppresses honey bees.
- Synergism between pesticide exposure and *Nosema* infections negatively impacted bee health.
- *Nosema* adversely impacts nutrient utilization.
- *Nosema* potentially interacts with other pathogens/parasites.
- Understudied pathogens and parasites that merit more research:
  - *Crithidia mellifica*, a trypanosome, is highly prevalent. Adverse effects by other species of *Crithidia* are known in bumble bees, and it seems likely that *C. mellifica* has negative effects on honey bees, at least in some circumstances.
  - *Spiroplasma* bacteria also occur in bees; possible seasonal effects on bee health.
  - Both positive and negative impacts of diverse digestive tract bacteria and other microbes on bee and colony health.
- Vigilance needed to prevent introduction of pests not yet detected in U.S.
  - Slow Bee Paralysis Virus
  - *Varroa spp.*
  - New strains of Thai Sacbrood Virus

## **Current State of Knowledge of Arthropod Pests and Best Management Practices**

*(Dr. Dennis vanEngelsdorp, University of Maryland, College Park, Maryland; and Dr. Jeff Pettis, USDA ARS, Beltsville, Maryland)*

### **Summary of Research Presentation:**

- Arthropod Pests in Bees:
  - Varroa mites (*V. destructor*)
    - Recognized as the major factor underlying colony loss in the U.S. and other countries, but is not associated as a primary factor in colony collapse disorder in the United States.
    - Immunosuppresses bees and vectors viruses that infect bees.
    - Has rapidly spread into Hawaiian honey bee colonies, despite the best efforts to control its spread.
    - There is evidence for widespread resistance to the chemicals used to kill mites (miticides), *e.g.*, fluvalinate and coumaphos, and a need for development of new effective treatments and alternative methods of mite control.
    - Other treatments that beekeepers have utilized do not appear to offer effective control or may have limited use.
    - The miticide, amitraz, may provide limited control due to developing resistance in Varroa, but data indicate that the amitraz formulation is important, as the formulation used in crop-pest control has increased toxicity to bees as opposed to the formulation intended for use in-hive (ApiVar<sup>®</sup>).
    - The adoption of bee stocks with behavioral resistance to Varroa has not been widely utilized.
    - New insights into Varroa may result from its genome having been sequenced (Cornman, 2010).
  - Tracheal mites (*Acarapis woodi*): Not widely detected nor regarded as a major factor in U.S. colony loss.
  - Small hive beetle can lead to increased colony loss via unknown mechanisms; use of in-hive small hive beetle traps results in significantly lower colony loss.

- Africanized bees continue to spread in the U.S. and have been permanently established in several states. To help impede additional spread, an improved identification system for Africanized bees is needed along with best management practices.
- Phorid flies are not considered to be a widespread problem or threat to colony health.
- Bee louse and wax moths are not of major concern at the current time.
- Vigilance needed to prevent introduction of pests and other bees and wasps not yet detected in U.S.
  - *Tropilaelaps* spp. (*T. clareae* and *T. koenigerum*) – parasitic mite (Asia)
  - Other bee subspecies and species: *A. mellifera capensis* (southern Africa), *A. ceranae* and *A. florea*
  - The Asian predatory hornet *Vespa velutina* (Asia, Europe)

### **Current State of Knowledge of Pesticides and Best Management Practices**

*(Dr. Reed Johnson, Ohio State University, Columbus, Ohio; Dr. James Frazier, Pennsylvania State University, University Park, Pennsylvania)*

**Summary of Research Presentation:** There is broad consensus among all stakeholders that pesticide use should not affect honey bees in such a way that 1. Honey production is reduced or 2. Pollination services provided by bees are threatened (Pesticide Risk Assessment for Pollinators Executive Summary, SETAC, 2011). However, it is not clear, based on current research, whether pesticide exposure is a major factor associated with U.S. honey bee health declines in general, or specifically affects production of honey or delivery of pollination services. It is clear, however, that in some instances honey bee colonies can be severely harmed by exposure to high doses of insecticides when these compounds are used on crops, or via drift onto flowers in areas adjacent to crops that are attractive to bees.

- For example, dust produced in the process of planting pesticide-coated seeds has been shown to contain high levels of insecticide with the potential to harm bees.

- Germany 2008: Seed treatment dust containing 12 to 28 percent clothianidin or thiamethoxam (Pistorius et al., 2009, 10th Int. Symposium of the ICP-Bee Protection Group)
- U.S. 2010: Talc containing 0.3 to 1.5 percent clothianidin or thiamethoxam (Krupke *et al.*, 2012, PLoS ONE)

It is also clear, based on chemical analysis of bees and bee products, that exposure of bees to a gamut of pesticides is commonplace, but the level of exposure to any particular pesticide is generally not enough to immediately or acutely kill bees (Mullin *et al.*, 2010).

- Traditional laboratory-based acute toxicity testing on adult workers (Tier 1), which determines LD<sub>50</sub> or LC<sub>50</sub> values, is required for registration of all pesticide testing.
- Acute toxicity testing does not test for effects beyond acute mortality and cannot detect any harm caused by pesticides that do not cause lethal effects, such as fungicides and herbicides.
- Acute toxicity testing cannot address sub-lethal insecticide effects on bees at levels too low to kill outright.
  - It is relatively straightforward to determine the level of pesticides contaminating both beehives and the environment. The most pressing research questions lie in determining the true pesticide exposure that bees receive and the effect, if any, that pervasive exposure to multiple pesticides have on the health and productivity of whole honey bee colonies. Determining the actual dose of pesticide that bees receive in ecologically relevant situations will help connect laboratory-based experiments using individual bees or bee tissues to expected pesticide effects in whole colonies. How are pesticides transferred to bees and exchanged between bees?
  - How do pesticides move within bees and the bee hive and how are these compounds metabolized and excreted by bees and bee colonies?
  - Which molecular receptors inside bees interact with pesticides?
- Can sublethal tests at the individual level predict effects on whole colonies?
  - Drones: Sperm number and viability/Longevity
  - Workers: Foraging success/Longevity

- Queens: Egg laying rate/Egg hatch/Longevity
- Many recent studies assessing sublethal effects in individual workers:
  - Proboscis Extension Assay (learning) (Ciarlo *et al.*, 2012)
  - Waggle dance behavior (Nieh *et al.*, 2012, J. Exp. Biol)
  - Sucrose responsiveness (Nieh *et al.*, 2012, J. Exp. Biol)
  - Mobility (Teeters *et al.*, 2012, Env. Tox. Chem.)
  - Foraging behavior - Short-term (3 hour) effects of neonicotinoids (Schneider *et al.*, 2012, PLoS ONE)
  - Forager loss - Henry *et al.*, 2012, Science; Predict effects of forager loss on colony growth using a demographic model (Khoury *et al.*, 2011, PLoS ONE)

Laboratory tests on individual honey bees have shown that field-relevant, sublethal doses of some pesticides have effects on bee behavior and susceptibility to disease. However, it remains a challenge to measure the effects of low-level, field-relevant exposure where it matters most: in real honey bee colonies. The social complexity of honey bees and the uncontrollable aspects of field research present substantial challenges to determining pesticide effects in whole-colonies. While experiments using whole colonies have the potential to directly address the effects of pesticides on honey production and pollination services, challenges presented by field or semi-field experiments include:

- Many colonies are needed per treatment due to high variability between honey bee colonies.
- The actual levels of exposure to pesticides that bees receive are still a big question.

Computer modeling of colony demographics following pesticide exposure shows promise in linking the results of laboratory-based pesticide studies with pesticide effects on whole-colony health.

- However, model predictions depend on the parameters used (Cresswell and Thompson, 2012, Science)
- Studies in progress seek to address this concern (Zhu *et al.* 2012 unpublished); model includes analyses of:
  - Food collection: number of foragers/food storagers
  - Queen egg-laying: queen fitness, brood care, available cells

- Brood: egg input, development
- Good hygiene
- Food storage: pollen, honey

An improved understanding of the physiological basis of pesticide toxicity in honey bees could lead to an understanding of the toxicity of pesticide mixtures and the potential interactions between pesticides and pathogens, nutrition and genetics.

- Interactions to be studied:
  - Pesticide-pesticide combinations are likely (Mullin *et al.*, 2010, PLoS ONE)
    - Average of 7.1 pesticides in pollen
    - Average of 2.5 pesticides in bees
  - Pesticide combinations can be more (or less) toxic (Johnson *et al.*, 2013, PLoS ONE)– need further research
  - Additive (pesticide 1 + pesticide 2)
  - Synergistic (pesticide 1 x pesticide 2)
  - Antagonistic (pesticide 1 – pesticide 2)
  - Miticide-drug interactions – Oxy-tetracycline, *tau*-fluvalinate (Hawthorne and Dively, 2012, PLoS ONE, multi-drug resistance transporters )
  - Many potential interactions remain to be explored: Pesticide-food/disease/season/temperature/age/genetics/management
- How can we better address the effects of pesticides on pollination and honey production?
  - Management
    - Improve communication between stakeholders
    - Further development of BMPs needed
    - Provide alternate forage
  - Continuing research being done:
    - Ecologically relevant dose
    - Modeling colonies
    - Sublethal effects
    - Interactions

## **Current State of Knowledge of Bee Genetics, Breeding, and Best Management Practices**

*(Dr. Marla Spivak, University of Minnesota, St. Paul, Minnesota; and Dr. W. Steve Sheppard, Washington State University, Pullman, Washington)*

### **Summary of Research Presentation**

- Historical pattern of honey bee introductions to the New World primarily occurred between 1622 and 1922. Eight Old World subspecies were introduced, including several from Africa, the Middle East and Europe. Only three European strains found favor with U.S. beekeepers: Italian, Carniolan, and Caucasian.
- Genetic diversity is critical to honey bees colonies
  - At the intra-colony level: genetic variation improves thermoregulation, disease resistance, worker productivity, *i.e.*, related to colony health
  - At the population level: U.S. honey bees show effects of multiple “bottlenecks”
    - Initial introductions of limited numbers of queens, queen production methods (One million queens produced from less than 600 “mother” queens),
    - highly restricted importation of new breeding germplasm since 1922
- Introduction of novel Old World genetic stock for breeding
  - USDA importation, selection and distribution of new stock (Russian Honey Bees)
  - WSU importation, selection and distribution of honey bee germplasm (semen) from original sources for three “favored” U.S. strains (Italian, Carniolan and Caucasian)
  - Recent development of practical means for long term storage (cryopreservation) provides means to store “top-tier” domestic honey bee germplasm for breeding use through “time and space” and to conserve germplasm collected from original source populations in Europe
- Bee Breeding – Cultural Shift
  - Growing interest to produce locally, regionally adapted strains of honey bees through small scale queen production
  - Driven in part by interest to breed bees more tolerant of mites and resistant to diseases and to reduce the amount of in-hive chemical inputs (miticides, antibiotics) needed to maintain healthy bees
- Tech Transfer Teams

- Assist the bee breeding industry incorporate objective selection-trait criteria in breeding
- Help implement scientific and technological advances to enhance sustainability and profitability
- Future of Bee Breeding
  - Marker-assisted selection (*i.e.*, selection of bees that possess genetic markers for desired traits).
  - Field assays for Varroa Sensitive Hygiene (VSH, a selectable trait whereby bees detect *Varroa* infested brood in capped cells and remove infested bee pupae, disrupting the mite's reproductive cycle) and other traits.
  - Increased baseline genetic diversity for trait selection.

## **Work Groups**

On the second day of the conference, invited participants were assigned to one of four work groups and invited to address questions developed by the Steering Committee and openly discuss facts, experiences, and viewpoints on one of the areas associated with honey bee health (Appendix 3). The sections that follow contain a summary of those work group discussions. *As noted earlier, the views expressed in this report are those of the presenters and do not necessarily represent the policies or positions of the U.S. Department of Agriculture, the Environmental Protection Agency or the United States Government.*

## **Nutrition**

The nutrition work group was chaired by Dr. Gloria DeGrandi-Hoffman (USDA ARS, Tucson, Arizona), and Dr. Nancy Moran (Yale University, New Haven, Connecticut). Dr. Mary Purcell-Miramontes (USDA NIFA) and Dr. Terrell Erickson (USDA NRCS) facilitated the discussion.

Questions developed to guide the discussion (Appendix 2) probed the nutritional composition of pollen before and after it is converted to bee bread and the contributions of beneficial microbes in metabolism, and food processing and storage for the hive. The discussion among stakeholders in the nutrition working group, though, primarily revolved around the shortage of high-quality forage for bees in the form of flowering plants, spatially and temporally. It was noted that availability of open foraging areas has declined drastically in the last few years, due to land use changes driven, in part, by economic and agricultural developments such as increased planting of row crops, such as corn, as commodity prices have risen, and in sites that formerly were undisturbed.

Although the purpose of this workgroup was not to recommend policies, some participants expressed the need for a land use policy that provides pesticide-free areas with blooming plants where beekeepers can safely place colonies. The primary point of this discussion was that beekeepers need access to more high-quality forage. Because of year-to-year weather fluctuations, forage areas should span a variety of regions and land types, particularly as parallels typical beekeeper migratory routes. For example, a drought in one part of the country can

drastically reduce the availability and quality of forage plants, and beekeepers need alternative sites and plants to cope with these fluctuations. Although diet supplements are essential for large-scale beekeepers, they are only a temporary substitute for high-quality floral resources. Therefore, good bee nutrition depends on how land around colonies is managed, and what flowers are available to bees.

The availability of diverse and nutritional forage was noted as being particularly important for building colony populations prior to and throughout pollination (especially of almonds) and afterward, because colonies need to recover from stresses associated with transport. Beekeepers remarked that colonies with access to good floral resources were generally healthier than those located where few floral resources exist (*i.e.* sites dominated by row crops) and fed dietary supplements. Undernourished or malnourished bees appear to be more susceptible to pathogens, parasites, and other stressors including toxins. Thus, nutrition might be a fundamental factor in mitigating negative effects of other stress factors on bee health. Issues related to Federal and State land management agencies, as well as policies or programs that affect land use and maintenance (such as The National Management Plan for Invasive Species), may be important considerations to bear on the issue of alternative forage.

It was apparent during the work group discussion that these are complex issues that will require the involvement of multiple agencies and individuals. Forage areas are impacted by various Federal and State agencies, individual landowners and growers. Management of these lands affects not only beekeepers, but also other interest groups, including environmentalists and sportsmen. There appeared to be wide agreement that solid research on the factors determining good bee nutrition will be an essential element for informing these decisions.

Questions and recommendations *generated by stakeholders* included the following:

- How much natural (or relatively unmanaged) forage is needed to support honey bees so that hives can produce surplus honey and provide vital pollination services?
- What are the benefits to agriculture (measured in increased yield) of having colonies near crops (such as soybeans and other crops not contracting for pollination services) if

increases in yield are realized, would this affect the attitudes and practices of growers in taking steps to mitigate potential risks to bee colonies in or near their fields?

- How do particular land management practices, from right of way management to existing and potential NRCS programming, or seed mixes affect bee nutrition and movement into adjacent cropping systems?
- Farm Services Agency Conservation Reserve Program Managers: Should Conservation Reserve Program consider alternatives to expensive seed mixes currently promoted (such as prairie grass/wildflower), toward less expensive mixes with legumes, which may give growers a greater incentive? Alfalfa could serve as a cheaper way of providing pollinator habitat. Development of a cost benefit analysis related to seed mixes used on Farm Services Agency Conservation Reserve Program Lands may provide insight on possible seed mix alternatives for these scenarios.
- How do particular supplements or other bee management practices affect nutrition? Stakeholders stated a need to understand how bee-associated microbes play a role in the nutrition of bees, potentially enabling them to make better use of particular foods.

### **Pathogens and Arthropod Pests**

Dr. Dennis vanEngelsdorp (University of Maryland, College Park, Maryland), Dr. Diana Cox-Foster (Pennsylvania State University, University Park, Pennsylvania), and Dr. Jay Evans (USDA ARS, Beltsville, Maryland) chaired discussion in the work group. Dr. Kevin Hackett (USDA ARS) and Dr. Robyn Rose (USDA APHIS) acted as facilitators.

There was general agreement that each question posed to guide the discussion (Appendix 2) regarding pathogens of honey bees was relevant, and further research is needed to develop solutions, but that some arthropod pests, such as small hive beetle, phorid flies, wax moth, and the bee louse, have less impact on colony health, and do not warrant increased research at this time. The group further agreed that additional information is needed about the biology of several pathogens and arthropod pests in order to develop new approaches to safeguarding honey bee health. In addition, the group recognized that new approaches to disease prevention and pathogen /arthropod pest introduction are urgently needed. Efforts toward research on disease

prevention should progress with as much synergism and coordination with international researchers and regulators as possible. These recommendations are summarized below.

*Biology of Pathogen and Arthropod Pests – Research Needs:*

- Develop a better understanding of interactions between honey bee symbionts, associated bee pathogens and arthropod pests.
- Pathogen and arthropod pests having major impact are in need of additional research, with Varroa being recognized of special concern, especially in association with viral diseases.
- Interaction of gut microbiome with immune systems in determining the outcome of pathogen infections needs to be better understood.
- Determine the mechanisms of pathogen and arthropod pest resistance to control tactics.
- Determine the basis for tolerance/resistance by the bees to the pathogens and arthropod pests.
- Determine the effects of different stresses (pesticides, nutrition, and climate) on disease biology in honey bees.
- Determine the role of arthropod pests in vectoring disease pathogens.

*New approaches to disease prevention and pathogen/arthropod introduction*

- Create a Diagnostics Decision Tree for disease diagnosis in honey bee colonies.
- Define the disease symptoms and develop a computerized diagnosis system that might be delivered as a smart phone app for use by beekeepers.
- Characterize symptoms of atypical death so it is immediately recognized and noted.
- Develop a standardized sampling method for different disease/arthropod pest symptoms
- Develop standardized diagnostics that have rapid turn-around.
- Create a centralized lab for diagnosis of samples submitted by beekeepers, researchers, and regulators.
- Develop methods to identify rogue variants or new virulent strains of pathogens/arthropod pests to allow for their rapid identification and response (see below).

*Management strategies for control of pathogens and arthropods – Research/Extension Needs:*

- Integrate disease surveys with surveys of management practices (*i.e.* Bee Informed survey).
- Improve integrated management tools (*e.g.*, monitoring tools) for pathogen and arthropod pest management.
- Develop new control measures for pathogens and arthropod pests, including new chemical approaches, traps, biocontrol, etc.
- Monitor for resistance in both arthropod and pathogen pests.
- Tailor approaches suitable for backyard bee keepers versus commercial operations.
- Develop novel dissemination tools (*e.g.*, smartphone apps).

*Surveillance – Research/Extension Needs:*

- Link and sustain different efforts that monitor bee health over time. Develop sampling methods of surveillance data to associate pest or pathogen levels with economic thresholds for bee colonies under different environmental regions of the U.S.
- Document, via surveys, as many pathogens and arthropod pests as possible, with integration of other data, such as: management strategies and control measures, nutritional state, pollen sources, crop / pesticide use in area, and climatic conditions.
- Determine what time of year works best for surveys, ideally having more than one survey/yr.
- Use the survey data to develop prediction models of bee mortality.
- Develop targeted surveys, including ports of entry with establishment of surveillance apiaries and swarm capture systems, for early detection of new arthropod and pathogen pests.
- Monitor for resistance to treatments in arthropod and pathogen pests.

*Develop a rapid response network for new pathogen and arthropod pest threats*

- Establish risk assessment methodologies for all known pathogen and arthropod pests.

- Create a national committee that can more rapidly respond and have the authority to carry out actions to protect honey bee colonies and the pollination industry.
- A response plan is needed that may include quarantines for newly introduced exotic pests (e.g., *Tropilaelaps*) and/or BMPs that may include destruction of infected/infested apiaries. A participant stated that it is essential that the plan include guaranteed measures for the financial compensation of beekeepers and efforts to “restock” their apiaries with ‘clean’ colonies.
- Synergize with similar efforts being done internationally to address bee health issues in both research and regulation arenas.

### **Pesticides**

Dr. Reed Johnson (Ohio State University, Columbus, Ohio) chaired discussion in the pesticides work group with facilitation provided by Dr. Tom Steeger and Tom Moriarty (U.S. EPA, Washington, District of Columbia) and Terry Anderson (Consultant, ARS, Beltsville, Maryland).

A central theme throughout the work group session was the need for informed and coordinated communication/education/extension of growers and beekeepers and the need for effective collaboration between stakeholders.

*(Pesticide Work Group Discussion Questions are in Appendix 2)*

### ***Best Management Practices***

- Beekeepers noted that moving colonies, placed in or near crops prior to pesticide application can reduce the negative effects of pesticides to colonies; however, depending on the season, it can be difficult to move colonies of differing sizes/weights and it can be difficult to locate suitable places to which to move the colonies.
- Altering colony locations can result in reduced homing success by forager bees.
- Commodity group representatives noted that some growers don’t require pollination services and that a knowledge gap exists between growers who need to treat pests quickly on a non-commercially pollinated crop and the potential presence of bees in the vicinity of these fields.

- Drift-Watch™ (<http://www.driftwatch.org>; recently renamed as FieldWatch™), a web-based tool to help identify where and when pesticide spray operations are occurring, and the process of registering the location of beehives were discussed as ways of identifying apiary locations and promoting communication with growers. However, it was noted that in certain existing state pesticide regulation programs that do use registries (such as Drift Watch) notification (of beekeepers) is recommended 48 hours prior to application, which may not provide enough time to move colonies.
- Another concern expressed is that beekeepers on contract to one grower may not be protected by pesticide applications to crops in adjacent fields. Beekeepers may receive notification from the adjacent grower, but the beekeepers may not be able to relocate their colonies because of their contract to provide pollination services.
- The use of repellents to deter bees from foraging in crop areas treated with pesticides was discussed. Some research has been done in the area of repellants, but participants stated more research is needed.
- Beekeepers expressed concern about hive placement in relation to needs of sufficient water and floral sources.
  - Supplemental diets (sugar/pollen/pollen substitutes) and supplemental water are potential means of providing uncontaminated sources of nutrition and water for bees.
  - Beekeepers reiterated the need to develop appropriate forage areas for bees.
- Night application was identified as a potential option for growers to reduce the risk of bee exposure to pesticides.
  - Better communication/education on proper application procedures could serve as an incentive for growers to apply pesticides with short residual toxicity at night.
  - Beekeepers also are doubtful that nighttime application of fungicides is an effective risk mitigation measure for honey bees.
- Some beekeepers raised concern that fungicides don't contain pollinator language on their labels, resulting in growers and beekeepers being uninformed about potential effects on bees.
- One participant suggested that education/mitigation efforts might focus on specific crops that pose the greatest risk to pollinators, although identifying particular crops as problematic may create divisions with growers who rent land to beekeepers.

- Participants recommended providing pesticide residual toxicity ( $RT_{25}$ , the time required for 25 percent bee mortality based on the test bee population exposed to the formulated pesticide product applied to foliage) data as a label advisory to improve pollinator protection.
- “Bee safe” labeling (*i.e.*, applying a “bee friendly” logo to certain product), which has been successfully instituted in France, was mentioned as a possible incentive for industry.
- One participant noted that when EPA’s registration process includes uncertainties (*e.g.* regarding potential effects), the burdens of which are unfairly born by the public. EPA therefore, should better account for potential risks before registering a pesticide. The participant also commented that EPA should have a better understanding of beekills; however, beekills often go unreported by beekeepers. (EPA is currently developing guidance to better standardize beekill investigations).

#### *Communication/Education*

- Extension information such as the Bee Informed Partnership (<http://beeinformed.org/>), which uses survey information collected directly from beekeepers, continue to be developed.
- There was a general sense that universities are developing materials on BMPs and pollinator protection; however, these materials may not be adequately distributed.
- A representative of Health Canada’s Pest Management Regulatory Agency (PMRA) described the process in which apiarists are assigned to each Province to work collaboratively with provincial departments of agriculture, grower groups, apiarists, and beekeepers in Canada.
  - Crop guidance documents prepared by provinces have pollinator information that is informed by PMRA risk assessments.
  - Beekeepers contact provincial officials to report beekill incidents and to obtain additional information; however, they do not report these incidents directly to federal officials.
- Commodity group representatives in the U.S. indicated that crop advisors are reliable sources of information. Commodity group representatives also noted that:

- U.S. growers may not be aware of the affects their activities may have on bees, and stated that most growers would willingly act to protect bees, if provided the proper education.
- Commenting on the fact that relationships are often at the base of rural agriculture, growers report a range of experiences when working with beekeepers.
- Many growers are unaware of potential risks associated with the newer pesticide chemistries, particularly newer compounds such as the neonicotinoids that were promoted as reduced-risk (*e.g.*, overall lower impact on human health and the environment) after passage of the Food Quality Protection Act (1996).
- Growers/applicators may not be reading labels, and their primary information for pesticide comes verbally from crop advisors, who may not be well-informed of the potential effects of newer chemistries on bees.
- It was suggested that communications between growers and their crop advisors should include information-sharing with beekeepers, current examples include:
  - Industry organizations, such as CropLife America and Responsible Industry for a Sound Environment (RISE, an affiliate of CropLife America working in urban environments), have included sessions on pollinator issues in annual meetings with their constituents.
  - The California Department of Pesticide Regulation has initiated efforts to have pollinator protection-related materials included in Pest Control Advisor certification course work.
  - Crop advisors recognize the limited time growers may have to discuss treatment options, and so discussions and, meetings are frequently conducted at edge of field. Situations such as this highlight the dynamic and sometimes difficulty in consulting with beekeepers prior to pesticide application.
- Web tools were mentioned as a means of distributing regionally specific information.
- Integrated pest management centers were also identified as sources of information about alternatives to pesticides?
- Leaflets developed by the French Ministry of Agriculture to describe their registration process were discussed as an example of how certain types of management information can be distributed.

- Beekeepers suggested that they have good relationships with growers and extension agents; subscribe to various list serves to obtain crop-specific information to stay abreast of emerging treatment options they may face when their colonies are in close proximity to these crops.
- Participants indicated the need for improving the knowledge of crop advisors in bee protection practices, as well as crop protection practices.
- Webinars, blogs, list serves, social media (*e.g.*, Twitter, Facebook) and commodity-specific newsletters, especially for crops not commercially pollinated (*e.g.*, grains), were discussed as a means of distributing information.

### *Regulatory*

- The importance of beekill reports and how they inform pesticide risk assessments was discussed.
- Accurate and timely beekill incident reporting, monitoring, and enforcement were identified as important.
  - Some beekeepers have been reluctant to report incidents for fear of damaging relationships with growers on whom they depend for pollination service contracts or honey production.
  - Concerns were expressed regarding beekeeper fear of retribution and/or distrust of government agencies they fear may cite them for illegal pesticide use for treatment of in-hive pests.
- Funding limitations have resulted in many States eliminating apiary inspectors and have also reduced extension efforts.
- Funding limitations have resulted in many States eliminating apiary inspectors and have also reduced extension efforts. This reduction in resources has led to loss of expert knowledge, thereby resulting in gaps in communication between beekeepers and growers. Stakeholders identified the need for a national coordinator that, among other things, would facilitate the dissemination of information to and between parties.

### *Research Needs/Funding*

- Research funded by commodity groups may yield information that is not widely disseminated beyond specific, and often poorly attended, commodity group meetings.
- Beekeepers noted the need for suitable forage areas to protect bees from pesticides.
  - Minimizing bee movement from bee yards into pesticide-treated crop land with use of forage plantings preferred by bees – research into size and composition of plantings.
  - Rights-of-way management that provides beneficial pollinator habitat – moves away from use of herbicides.
  - Land managers expressed concerns that invasive weed control efforts may be reducing the amount and diversity of available pollinator forage areas, particularly as associated with Rights-of-Way management.
- Stakeholders noted the need to identify “drivers” that make a difference in risk management/assessment; these may best be identified through the use of some of the forecasting models that have been developed with specific measurement end points that have the greatest effect on colony survival.
- Concern was expressed that land-grant scientists do not have incentives to engage in applied research that does not contribute to tenure/publications. Participants stated that such incentives should be increased to encourage researchers to further develop practices that mitigate the risk of bees to pesticide exposure.
- Funding mechanisms need to be identified that may allow government and university researchers to seek funds provided through industry/commodity groups without appearance of undue influence (*e.g.*, possible use of 501c (3) organizations).
  - University researchers reported reluctance in accepting any money from industry because outside observers may question whether the resulting research was biased.
- Credibility issues were identified with industry-funded research supporting pesticide registration.
  - An opinion was expressed that the EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel selection process is biased by avoiding any associations with industry

- EPA personnel noted that under the FIFRA, the regulated community is required to provide data to support the registration of pesticides and that this burden for generating such data does not fall on taxpayers.
- Participants stated there is a need for applied research in addition to basic research to address some of the questions regarding BMP development and efficacy evaluations.

### **Bee Genetics, Breeding, and Biology**

The bee genetics, breeding, and biology work group discussion group was chaired by Dr. Marla Spivak (University of Minnesota, St. Paul, Minnesota) and Dr. W. Steve Sheppard (Washington State University, Pullman, Washington), and facilitated by Dr. David Epstein (USDA OPMP, Washington, District of Columbia).

*(Genetics/Breeding/Biology Work Group Questions in Appendix 2)*

At the outset, the work group participants noted that long-term, sustainable solutions for honey bee health and productivity issues would most likely derive from selective breeding and genetic improvement of honey bees. A strong and healthy population of managed honey bees is required to provide pollination services for the agricultural sector, an activity critical to U.S. food security.

**Breeding Populations:** Honey bees are not native to North America, and our current managed honey bee population reflects the genetic consequences of historical introductions that included representatives from only 25 percent of the described honey bee subspecies in the Old World. Recent research indicates that North American honey bees show evidence of admixture among some of the subspecies sampled in these early introductions, a feature that may have helped U.S. honey bees avoid inbreeding-related problems (Delaney *et al.* 2009, Harpur *et al.* 2012, Sheppard 1989). As is typical with other agricultural animals and crops with Old World origins, sources of novel germplasm and genetic diversity for long-term breeding efforts can be secured through importation, quarantine, and screening of genetic material from areas of original distribution.

#### Breeding Tools/Trait Selection:

- Emphasis is on selection for increased hygienic behavior in commercial strains of bees.
  - Colonies expressing high levels of hygienic behavior show improved resistance to Varroa mites and diseases, such as American Foulbrood
- Work group participants indicated that a number of additional traits would be useful to include in breeding efforts and called for the development of both marker-assisted selection and improved field assays for testing various traits.
- The following traits were discussed; group consensus was that significant progress toward incorporation of these traits in breeding efforts might be expected in the short term:
  - *Varroa-Sensitive Hygiene (VSH)*
  - *Grooming Behavior (against Varroa mite)*: whereby mites are physically removed from infested adult bees, or sometimes killed by chewing (highly expressed in the species, *A. ceranae*; original host of Varroa). Several labs are working on this trait; progress has been made toward identifying genetic markers that may be used to assist breeding.
  - *Chalkbrood resistance*: Developing assays to select for resistance to this fungal disease in honey bee populations would be useful.
- Bee strains are available that express the above listed traits. However, participants recommended that association studies between traits and genetic markers be conducted, with the ultimate goal being marker-assisted selection. The rationale of this approach is that the ability to select for desirable traits within current commercial queen producer stocks would be most likely to engender acceptance among queen producers and also permit the rapid dissemination of these traits into the wider U.S. honey bee population.
- There was a general discussion of specific commercial strains of honey bees that have an innate resistance to various parasitic mites, particularly the “Russian honey bee,” originating in Russia and imported by the Baton Rouge Agricultural Research Service lab. The consensus was that current commercial production of this strain (approximately 2,000 queens per year) was not likely to make a significant genetic impact on overall commercial production of queens in the United States (approximately 1 million queens annually). However, specific traits associated with this strain (mite tolerance) and with USDA-developed VSH bees (mite resistance) are highly desirable and an effort should be

made to select for or otherwise incorporate similar traits within the U.S. breeding population.

- Germplasm Repository: The recent development and improvement of cryopreservation methods for honey bee semen provides significant opportunities for honey bee breeders. Workshop participants discussed the potential importance of establishing a honey bee germplasm repository and supporting research on honey bee cryopreservation to evaluate storage characteristics and limitations. Cryogenic reproductive technology is widely used in breeding programs with a number of agricultural animals (e.g., turkeys, sheep, beef and dairy cattle, and swine) and has been responsible for significant improvements in measures of stock productivity where it has been introduced. Cryogenic preservation of bee germplasm resources provides both a means to address conservation needs and practical breeding goals.
  
- A honey bee germplasm repository would serve as a place to maintain (for many years or decades) novel honey bee germplasm of three subspecies (*A. mellifera ligustica*, *A. mellifera carnica*, and *A. mellifera caucasica*) currently being imported from the Old World under a permit granted by the Animal and Plant Health Inspection Service.
  - At present, aliquots of this semen are being used to inseminate U.S. queen bees for release in a collaborative project with western U.S. queen producers and others are maintained in cryogenic storage as part of a long-term breeding program.
  
- A germplasm repository would allow for the preservation of “top tier” domestic genetic resources from the current U.S. honey bee population.
  - Queen producers could cryopreserve examples of their best lines of honey bees and then, years or decades later, retrieve these from liquid nitrogen storage to backcross to extant populations.
  - Such a repository effectively provides the option for queen producers to breed across time (different year classes) and space (easy transportation of genetic material) in ways previously unavailable.
  
- Technology Transfer Teams: Workshop participants also discussed the establishment and support of Tech Teams regionally within the U.S. to assist beekeepers. The system discussed was based on a model in use in Canada and another one currently operating in

California to assist queen breeders. The concept of the Tech Team is that a group of trained individuals work in the field with beekeepers to assess stocks and provide information that would inform management decisions to assess and breed bees (in the case of queen producers) or maintain colony health (all locations). The approach represents a new, field-active model of extension and a tool for action at the interface of science (applied research) and industry (informed management). The model calls for a fee-for-service approach that will make the tech teams self-supporting within a few years.

- The Tech Team currently assisting California queen bee breeders provides data that allows producers to assess their genetic stocks for specific traits of interest (hygienic behavior, for example). The teams are in place to also provide selection assistance to breeders as other traits become available. The Tech Team approach provides a means to incorporate objective criteria into the breeder's traditional process of choosing breeding queens.
- In addition to assisting the industry with the implementation of research findings such as genetic improvement (in California) or colony health (California, and Midwestern and Southeastern states), Tech Teams also provide a means for capturing data on current honey bee populations that can be used for epidemiological analyses or breeding (through identification of high-quality stocks).
- A new Tech Team is starting up in the Midwest as part of the Bee Informed Partnership (<http://beeinformed.org/>), and there is strong interest to develop a Tech Team for the southeastern United States.
- Diagnostic Laboratories: Few diagnostic laboratories are available in the United States to support beekeepers that wish to submit samples of their bees for determination of pathogen and parasite loads. Work group participants discussed the utility of establishing one or more diagnostic laboratories tasked with providing rapid turnaround analyses of pathogens and parasites for Tech Teams and beekeepers.
- The primary organisms that need to be analyzed by diagnostic laboratories include *Nosema spp.*, *V. destructor*, and tracheal mites (*Acarapis woodi*), although these laboratories could also be useful in evaluating submitted stocks for genetic markers for trait selection, as that technology becomes available.
- In areas where Africanized honey bees occur, there would also be a demand to analyze samples to determine the extent of genetic introgression from Africanized honey bees.

- Other Issues: The following additional topics were noted by participants as needing additional research:
- Queen failures: There is a widespread perception that honey bee queens do not live as long as they used to. Research into the possible causes of early supersedure, the process by which one queen bee is replaced by a new queen, or queen failure without replacement is needed to determine prevalence and causes, such as genetics, pathogen, pesticides, nutrition, management, and shipping.
- Genetically based treatments for pathogens: Interference RNA (RNAi), technology research is needed on honey bees and other pollinators. RNAi is a process used by many different organisms to regulate the activity of genes, and is also known as post-transcriptional gene silencing.
- Signaling and communication: Basic research is needed to understand signaling and communication within the colony and between pests and bees.
- Lack of research funding for applied bee issues: Work group participants also discussed the difficulty that researchers have in finding adequate funding to carry out studies in applied bee research. Participants recommended exploring whether USDA could develop a call for proposals on applied issues in colony health and beekeeping sustainability.

**Conclusion**

Overall and consistent with the stated objective, this conference provided an overview of a significant body of new knowledge on the current state of the science of honey bee health to the CCD Steering Committee that will be helpful in updating the CCD action plan. Stakeholders also identified a number of BMPs to potentially address factors associated with declines, and research needs were clearly articulated as well toward addressing uncertainties. In response to stakeholder input provided at the conference and based on the available science and its associated uncertainties, the CCD Steering Committee will revise the CCD action plan. The purpose of the action plan is to synthesize current recommendations from stakeholders and to coordinate an updated Federal strategy to address honey bee losses. The decline of honey bees and other pollinators continues to be a high priority topic for the USDA and the U.S. EPA. Intramural and extramural research and extension to elucidate the factors associated with losses and mitigating risks remains a high priority. We anticipate that the next CCD action plan will be completed in 2013 to early 2014.

**Acknowledgment**

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## Appendix 1. Conference Agenda

### National Stakeholders Conference on Honey Bee Health

October 15-17, 2012

Sheraton Suites Old Town Alexandria, 801 North St. Asaph Street, Alexandria, Virginia

Agenda: Day 1, October 15, 2012

#### Plenary session

8:00AM – 8:30AM: Opening Remarks: USDA Deputy Secretary, Kathleen Merrigan; U.S. EPA Deputy Administrator, Bob Perciasepe.

8:30AM – 8:40AM: Welcome, Sonny Ramaswamy, USDA NIFA Director

8:40AM – 9:30AM: Keynote Speaker, May Berenbaum, University of Illinois, Urbana-Champaign: Overview of the State of Our Pollinators

9:30AM-10:40AM: Stakeholder Opening Comments

*Presenters:* Darren Cox, Beekeeper Representative to US EPA Pollinator Program Dialogue Committee; Dan Botts, Minor Crop Farm Alliance; Gabrielle Ludwig, Almond Board of California; Barbara Glenn, Senior VP, Science and Regulatory Affairs, CropLife America; Laurie Davies Adams, *Executive Director*, North American Pollinator Protection Campaign; Christi Heintz, Apis m

10:40AM – 11:00AM: Break

#### Topic Presentations:

11:00AM – 11:30AM: Current State of Knowledge of CCD and its Relation to Honey Bee Health; Jeff Pettis, USDA ARS; Dennis vanEngelsdorp, University of Maryland

11:30AM – 12:00AM: Current State of Knowledge of Bee Biology; Jay Evans, USDA ARS

12:00PM – 1:30PM: Lunch

1:30AM – 2:00PM: Current State of Knowledge of Nutrition and Best Management Practices; Gloria DeGrandi-Hoffman, USDA ARS, Tucson, Arizona; Nancy Moran, Yale University

2:00PM – 2:30PM: Current State of Knowledge of Pathogens and Best Management Practices; Diana Cox-Foster, Pennsylvania State University; Jay Evans, USDA ARS

2:30PM – 3:00PM: Current State of Knowledge of Arthropod Pests and Best Management Practices; Dennis vanEngelsdorp, University of Maryland; Jeff Pettis, USDA ARS

3:00PM – 3:30PM: Break

3:30PM – 4:00PM: State of Knowledge of Pesticides and Best Management Practices; Reed Johnson, Ohio State University; Jim Frazier, Pennsylvania State University

4:00PM – 4:30PM: Current State of Knowledge of Bee Genetics, Breeding and Best Management Practices; Marla Spivak, University of Minnesota; Steve Sheppard, Washington State University

4:30PM – 5:00PM: Break

5:00PM – 7:00PM: Evening Discussion/Networking Session – Transition to Day 2 Work Group Sessions

Day 2, October 16, 2012

8:00AM – 10:00AM: Work Group Sessions

- Nutrition
- Pathogens and Arthropod Pests
- Pesticides
- Bee Genetics, Breeding, Biology

10:00AM – 10:20AM: Break

10:20AM – 12:00PM: Work Group Sessions

- Nutrition
- Pathogens and Arthropod Pests
- Pesticides
- Bee Genetics, Breeding and Biology

12:00PM – 1:30PM: Lunch

1:30PM – 3:10PM: Conference participants reconvene in general session

- Work Group Reports to the Whole:

1:30 – 2:15: Nutrition

2:15 – 3:00: Pests

3:00PM – 3:20PM: Break

3:20PM – 5:00PM: Conference participants reconvene in general session

- Work Group Reports to the Whole:

3:25 – 4:10: Bee Genetics, Breeding, Biology

4:10 - 5:00: Pesticides

Day 3, October 17, 2012

8:30AM – 10:00PM: Federal CCD Steering Committee meeting with research leaders to summarize conference input.

10:00 AM – 12:00PM: Federal CCD Steering Committee meeting to revise Federal CCD Action Plan.

Conference Steering Committee:

David Epstein, USDA OPMP; Tom Moriarty and Tom Steeger, US EPA; Kevin Hackett, USDA ARS; Robyn Rose, USDA APHIS; Mary Purcell-Miramontes, USDA NIFA; Terrell Erickson, USDA NRCS.

## Appendix 2. Questions Developed for Day 2 Work Groups.

### *Questions developed for discussion in the Nutrition Work Group:*

- 1) How do we evaluate the nutritional value of pollen? How does the nutritional composition of pollen change after it is converted to bee bread?
- 2) How do protein and carbohydrate supplements affect beneficial gut microbes?
- 3) Does pollen and nectar contamination with pesticides/fungicides affect beneficial microbes in stored pollen and the bee's digestive system?
- 4) Is there an interaction between nutritional status in a colony and its susceptibility to disease and parasites? Is a colony's response to treatments for foulbrood, Nosema or mites affected by nutrition?
- 5) How can we balance treatments so that beneficial microbes are not negatively affected, while still controlling pathogens and pests?
- 6) Has anyone noticed apparent detrimental effects from treatments with Tylosin®, Terramycin®, or other anti-microbials?
- 7) What research projects would add most information to understanding how microbes in colonies are affecting colony health?

### *Questions developed for discussion in the Pathogens Work Group:*

- 1) What are the best ways to describe a pathogen/disease so that others can determine if they have the same organism?
- 2) What are the health impacts of 'neglected' parasites/pathogens/or potential symbionts like Crithidia, fungi, amoebae, lactobacteria, spiroplasma?
- 3) How best to sample, preserve and screen samples for disease both unknown and known?
- 4) How can surveys be better used to predict/mitigate disease (targets, time scales, costs)?
- 5) Which management processes are responsible for enabling disease spread/ minimizing disease occurrence?
- 6) Are other pollinator species also being impacted by viral infections and common stress factors?

- 7) How does Varroa increase virulence of transmitted viruses via impacts on bee immunity and impacts on viral load and 'readiness'?
- 8) Can a single method be developed to sample adult bees or brood that will work for most pests and pathogens or do we need specific sampling regimes for each?

*Questions developed for discussion in the arthropod pests Work Group:*

- 1) Can Varroa and European honey bees reach stable host-parasite equilibrium if we reduce chemical controls?
  - a. Do we have commercial stocks that are viable for pollination?
  - b. Are chemical treatments doing more harm than good?
- 2) Rank the following in terms of importance for dealing with Varroa new chemical controls, new biological controls, understanding of resistance mechanisms by Africanized and Asian honey bees, means to understand and disrupt the mite-virus interaction.
- 3) Are the current traps and chemical controls adequate for dealing with small hive beetle and if not what are areas of research that would be most helpful?
- 4) Have chemical treatments for Varroa made tracheal mites scarce or has natural selection driven tracheal mite levels down?
- 5) Are tracheal mites still an issue in bee health?
- 6) Should research be directed at novel or rare pests (*i.e.* phorid flies, the bee louse *Braula*, etc)?
- 7) Should research be conducted on known threats from abroad and if so rank the following? ( *Tropilaelaps* mites, *Apis cerana*, *capensis* honey bees and Thai sacbrood)
- 8) What do we need to know about the lifecycles of honey bee threats not found in the U.S. in order to be prepared for eradication efforts and/ or providing management advice in the event of their introduction?
- 9) Do we need new control methods for wax moths?
- 10) How best management practices are effectively disseminated through the beekeeping community?

*Questions developed for discussion in the Pesticide Work Group: Management Practices*

- 1) Best Management Practices:
  - a. Are there sources for grower/beekeeper BMPs that are currently in use (Project *Apis m*)?
  - b. What practices do beekeepers use or prefer in order to minimize the potential impact of pesticides to managed honey bees? Do practices differ by crop or region?
  - c. To what extent are growers aware of the potential impact their activity may have on bees?
  - d. What practices do applicators or growers use or prefer to minimize the potential impact of pesticides to managed honey bees?
- 2) Who/what are the best information sources for growers when choosing products to protect crops?
  - a. Do these sources (such as Pest Control Advisors (PCAs) have access to information on best management practices with respect to pollinator protection?
  - b. Do beekeepers consult with these sources (such as PCAs) or with growers to work out management practices that may present lower potential risk from pesticides? If not, why not?
- 3) What options are available to improve communication between stakeholders (state officials, growers, applicators and beekeepers improve risk management?
- 4) How can stakeholders (state officials, beekeepers, growers, and applicators) work together to build integrated plans to protect against pests insects and protect managed pollinators?
- 5) Are there efforts underway to develop Best Management Practices that apply to pesticide use in agricultural settings? Are there efforts underway to develop Best Management Practices that apply to in-hive use of pesticides? If so, what is likely to result from these efforts? If not, why not? Are there exposure scenarios or routes that stakeholders feel have not been identified by federal/state regulatory partners?
- 6) Does reserve/non-crop land provide a pesticide-free forage scenario for managed bees, and if not, why and how can it be managed?
- 7) How can a beekeeper know if pesticides exposure is a factor in colony loss or weakening?
  - a. At the colony level, how does acute exposure to a pesticide differ from that of chronic exposure?

- 8) Historically, what is the typical loss a beekeeper experiences from pesticides?
  - a. Aside from obvious losses, do beekeepers feel that delivery of pollination services and honey production have been affected by pesticide exposure?
  - b. Do beekeepers feel that current loss due to pesticides is equally associated across crops, or across the country? That is, do beekeepers feel that their losses from pesticides would be different if they worked in different states or contracted with different crops?
- 9) Do miticides cause losses? What is an acceptable level of loss due miticide exposure? Can beekeeping survive and be profitable without use of miticides?
- 10) To what extent are alternative forage areas a viable option in heavily developed agricultural areas?

*Questions developed for discussion in the Pesticide Work Group: Research*

- 11) Can we directly measure the effects of pesticide exposure on delivery of pollination services and honey production? Can other, more easily measured, endpoints be used to predict pesticide effects on pollination and honey production? Is colony strength an adequate measurement endpoint?
- 12) Can sublethal pesticide exposure be shown to affect pollination and honey production? How can we relate sublethal exposure effects of individual bees (PER, mobility, homing) to whole colony success? Can sublethal testing on individuals be improved?
- 13) How can we get to a better estimate of bees' pesticide exposure? Is it possible to estimate individual bees' body burden (the Ecologically Relevant Concentration) of pesticide through empirical measurement or toxicokinetic/toxicodynamic modeling?
- 14) Interactions could occur between insecticides, miticides, fungicides, herbicides, adjuvants, pests, pathogens, nutritional status, microbial community, plant xenobiotics, seasonality, management practices, caste, life stage and genetics. Some combinations are likely to be either harmful or beneficial to bees – how do we discover these without testing all possible combinations?
- 15) How do we pay for pesticide-related bee research?

*Questions developed for discussion in the Genetics/Breeding/Biology Work Group:*

1. Genetic Diversity: Genetic diversity of the honey bee may now be considered on a global scale. For example: the total diversity of managed “Italian” honey bees may be best represented by honey bees from Italy (the original subspecies) *and* managed populations in the Americas and Australia. All of these may be viable pools that could contribute to establishing populations for selective breeding. A cryogenic storage facility could maintain germplasm from both natural and managed honey bee populations for future breeding. Thus, in addition to Old World source populations, genetic samples of specific desirable commercial lines of bees could be placed into cryogenic storage for later recovery. Cryogenic storage addresses an overarching USDA mandate to preserve germplasm from animals and plants of agricultural significance: “The mission of the National Center for Genetic Resources Preservation (NCGRP) is to acquire, evaluate, preserve, and provide a national collection of genetic resources to secure the biological diversity that underpins a sustainable U.S. agricultural economy through diligent stewardship, research, and communication.” Despite initial efforts to sustain a honey bee stock center, the cost needed to maintain genetic diversity in large cohorts of living colonies was prohibitive. Now that functional cryopreservation technology is available, is it time to reconsider the *status quo*? That is: Is there a need for a major effort to establish a national honey bee germplasm repository?
  
2. Breeding – *Commercial bee breeding*: The goal of the tech-transfer “Bee Team”, funded by the Bee Informed Partnership and fees-for-service, is to work directly with bee breeders in California to improve stock selection, enhance genetic diversity, and engage in disease and parasite-related diagnostic evaluations. In addition to helping bee breeders keep track of and select colonies with the lowest mite, Nosema and virus levels, the Bee Team assists with selection for hygienic behavior using the freeze-killed brood assay.
  - a. What other traits could be selected? Are we ready to implement marker-assisted selection for grooming behavior and VSH (e.g., the Bee Team could send samples to a lab for genetic testing)?

- b. Toward a sustainable and diverse genetic base: What is the best way to incorporate additional imported honey bee genetic material into the actual breeding populations of the U.S.?

*Local/ regional bee breeding:* Many beekeepers would like to select for one or more of the following: “locally adapted” stock; survival stock; and/or stock that does not require any chemical treatments.

- a. How to balance genetic diversity and selecting for resistance, while trying to keep things locally adapted? What does “locally adapted” mean in terms of honey bees?
  - b. Given the perceived differences in selection criteria between large commercial interests and beekeepers working to develop locally selected populations, how do the roles of subspecies origin, selection criteria for pest and parasite control and overwintering strategies inform the choice of the initial population for breeding?
3. Queen Failures: Real or Perceived?
    - a. If real: Is this problem tied to race, stock, type of beekeeping operation, old vs. new combs? Is the problem caused by not enough time spent in mating nucs? Or pathogen (viruses, Nosema?) Or pesticide residue?
  4. How do we weigh the impacts of behavioral and physiological (immunity, development) traits on bee health? Similarly for individual and ‘social’ traits? There must be trade-offs for bees, in terms of costs of maintaining these traits, so we can’t just push them to be above average at everything.
  5. How can bee x bee and bee x pest signaling be exploited to 1) control pests, 2) manipulate foraging and other colony traits, 3) maintain respect for the queen?
  6. How can standards and protocols be normalized across labs and countries: controlled language, Beebook for protocols, true Standard Operating Procedures? These are all needed, especially with touchy regulatory issues.
  7. What is the current consensus on biological and abiological factors that act non-additively to impact bee health, do any cancel each other out or is it always  $1 + 1 \geq 2$ ? How do we use this knowledge (*e.g.*, are survey tools economic for making management decisions? Can knowing that certain factors interact negatively for bees be used to more strongly regulate those factors when they are likely to co-occur?

8. Six years post-honeybee genome what have we learned about bee biology and what is in place for the major questions of breeding, vetting traits, parasite interactions, novel controls (RNAi), and management to make bees less stressed and more productive.
9. Six years post-CCD what have we learned from the added efforts put into bee disease and responses to chemical stresses? Which new tools or ways of thinking/models are going to change the field and improve bee health?
10. Will the world accept genetic strategies from i) RNAi versus pests, ii) RNAi to influence bee behaviors? iii) germline transformation of bees even if it tackles a critical weak point like viral resistance?

**Appendix 3: Invited Work Group Participants**

(The participant lists in this appendix do not represent all who actually participated in each work group. Some invitees did not attend the conference. Note that recorders are not listed.)

**Invited Work Group Participants - Nutrition****Leaders**

Degrandi-Hoffman, Gloria	USDA ARS
Erickson, Terrell,	USDA NRCS
Moran, Nancy,	Yale University
Purcell Miramontes, Mary	USDA NIFA

**Participants**

Berger, Lori	California Specialty Crop Association
Browning, Zach	Browning Honey Co. Inc.
Davies-Adams, Laurie	North American Pollinator Protection Campaign
Delaney, Deborah	University of Delaware
Downey, Danielle	Apiary Inspectors of America
Hayes, Jerry	Monsanto/Beeologics
Heintz, Christi	Project Apis m
Johnson, Jody	Smithers Viscient
Kelly, Iain	Bayer CropScience
Kuivila, Kathryn	USGS
Ludwig, Gabrielle	Almond Board of California
Overmyer, Jay	Syngenta Crop Protection
Verhoek, Randy	Harvest Honey Inc ND, NHBA
Wehling, Wayne	USDA APHIS

**Unable to Attend**

Eischen, Frank	USDA ARS
Esaias, Wayne	UMD/NASA (emeritus)
Haun, Gray	Tennessee Department of Agriculture
Hyberg, Skip	USDA FSA
Mussen, Eric	California Department of Food and Agriculture
Rao, Sujaya	Oregon State University
Sanroma, Joe	LA Beekeepers Association, AHPA
Trumble, John	UC Riverside
Tucker, Tim	Tucker Bees, Kansas, ABA
Vaughn, Mace	Xerces Society
Wardel, Gordy	Paramount Farms, CA

**Invited Work Group Participants – Pathogens/Arthropods**

**Leaders**

Cox-Foster, Diana	Pennsylvania State University
Evans, Jay	USDA ARS
Hackett, Kevin	USDA ARS
Rose, Robyn	USDA APHIS
vanEngelsdorp, Dennis	University of Maryland

**Participants**

Abbott, John	Syngenta Crop Protection
Burand, John	University of Massachusetts
Caron, Dewey	University of Delaware
Cox, Darren	Cox Honeyland, NHBA
Coy, Steve	Coy's Honey Farm
Cruise, Chris	Mann Lake Beekeeping Supplies
Feken, Max	Florida Dept of Agriculture and Consumer Services
Glenn, Barb	CropLife America
Hackenberg, David	Hackenberg Apiaries, ABF
Huang, Wei-Fone	University of Illinois
James, Rosalind	USDA ARS
Meikle, William	USDA ARS
Rogers, Dick	Bayer CropScience
Skinner, John	University of Tennessee
Smallwood, Ben	USDA NRCS
Stewart, Colin	USDA APHIS
Stoner, Kimberly	Connecticut Agricultural Experiment Station
Teal, Peter	USDA ARS
Webster, Tom	Kentucky State University
Westervelt, David	Florida Dept of Agriculture and Consumer Services

**Unable to Attend**

Averill, Anne	University of Massachusetts
DiSalvo, Carol	National Park Service
Haterius, Stephen	National Association of State Departments of Agriculture
Kozak, Paul	Canadian Association of Professional Apiculturists
Kramer, Vince	Dow AgroSciences
Levi, Ed	Arkansas State Plant Board

**Invited Work Group Participants - Pesticides**

**Leaders**

Johnson, Reed,	Research Leader, Ohio State University
Steeger, Tom,	Facilitator, U.S. EPA Office of Pesticide Programs
Moriarty, Tom,	Facilitator, U.S. EPA Office of Pesticide Programs
Anderson, Terry	Facilitator, USDA ARS

**Participants**

Adee, Bret	American Honey Producers Association
Alix, Anne	Dow AgroSciences
Berenbaum, May	University of Illinois, Champaign-Urbana
Bireley, Richard	California Dept. of Pesticide Regulation
Christiansen, Jessica	Monsanto/Beeologics
Egan, Peter	Armed Forces Pest Management Board
Fischer, David	Bayer Crop Science
Hansen, George	American Beekeepers' Federation
Harriot, Nichelle	Beyond Pesticides
Hart, Connie	Canada Pest Management Regulatory Agency
Hooven, Louisa	Oregon State University
Hou, Wayne	Canada Pest Management Regulatory Agency
Johansen, Erik	Washington State Department of Agriculture
McCain, Pat	Syngenta Crop Science
Mendes, Dave	Commercial Beekeeper, FL
O'Neill, Bridget	DuPont Chemical
Parker, Don	National Cotton Council
Pettis, Jeff	USDA ARS
Ruckert, Ed	McDermott Will & Emery LLP
Seetin, Mark	U.S. Apple Committee
Tindal, Nick	Association of Equipment Manufacturers
Trainer, Maria	CropLife Canada

Walker, Larissa Center for Food Safety  
Wisk, Joe BASF

**Unable to Attend**

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Lu, Chensheng Harvard University  
Rowe, Brian Michigan Department of Agriculture  
Sass, Jennifer Natural Resources Defense Council  
Tignor, Keith Apiary Inspectors of America  
Willet, Mike Northwest Horticultural Council  
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Sheppard, Steve	Washington State University
Spivak, Marla	University of Minnesota

**Participants**

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Boess, Bruce	USDA NASS
Culiney, Tom	USDA APHIS
Danka, Robert	USDA ARS
de Guzman, Lilia	USDA APHIS
Dolezal, Adam	Iowa State University
Dykes, Mark	Florida Dept of Agriculture and Consumer Services
Hawthorne, David	University of Maryland
Henderson, Colin	Bee Alert Technology
Mattila, Heather	Wellesley College
McCallister, Ray	CropLife America
Pruisner, Robin	Iowa Dept of Agriculture
Rinderer, Tom	USDA ARS
Rouse, Gus	Kona Queen Hawaii, Inc
Rueppell, Olav	University of North Carolina
Tarpy, Dave	North Carolina State University
Zisook, Elsa	Valent U.S.A. Corporation

**Unable to Attend**

Bourgeois, Lanie	USDA ARS
Buchman, Steve	Pollinator Partnership
Cane, Jim	USDA ARS
Derisi, Joe	University of California San Francisco

Sagili, Ramesh  
Weaver, Danny

Oregon State University  
BeeWeaver Apiaries, TX

#### Appendix 4. Cited References for Biology Research Presentation

- Alaux, C., Brunet, J. L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L. P., and Le Conte, Y. (2010). Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environmental Microbiology* 12(3), 774-782.
- Alaux, C., Dantec, C., Parrinello, H., and Le Conte, Y. (2011). Nutrigenomics in honey bees: Digital gene expression analysis of pollen's nutritive effects on healthy and Varroa-parasitized bees. *BMC Genomics* 12.
- Ament, S. A., Wang, Y., Chen, C., Blatti, C. A., Hong, F., Liang, Z. S., Negre, N., White, K. P., Rodriguez-Zas, S. L., Mizzen, C. A., Sinha, S., Zhong, S., and Robinson, G. E. (2012). The transcription factor ultraspiracle influences honey bee social behavior and behavior-related gene expression. *PLoS Genetics* 8(3).
- Anderson, K. E., Sheehan, T. H., Eckholm, B. J., Mott, B. M., and DeGrandi-Hoffman, G. (2011). An emerging paradigm of colony health: Microbial balance of the honey bee and hive (*Apis mellifera*). *Insectes Sociaux* 58(4), 431-444.
- Calderón, R. A., Fallas, N., Zamora, L. G., van Veen, J. W., and van Sánchez, L. A. (2009). Behavior of Varroa mites in worker brood cells of Africanized honey bees. *Experimental and Applied Acarology* 49(4), 329-338.
- Cornman, R. S., Tarpy, D. R., Chen, Y., Jeffreys, L., Lopez, D., Pettis, J. S., vanEngelsdorp, D., and Evans, J. D. (2012). Pathogen webs in collapsing honey bee colonies. *PLoS ONE* 7(8).
- Engel, P., Martinson, V. G., and Moran, N. A. (2012). Functional diversity within the simple gut microbiota of the honey bee. *Proceedings of the National Academy of Sciences of the United States of America* 109(27), 11002-11007.
- Evans, J. D., and Spivak, M. (2010). Socialized medicine: Individual and communal disease barriers in honey bees. *Journal of Invertebrate Pathology* 103(SUPPL. 1).
- Foret, S., Kucharski, R., Pellegrini, M., Feng, S., Jacobsen, S. E., Robinson, G. E., and Maleszka, R. (2012). DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees. *Proceedings of the National Academy of Sciences of the United States of America* 109(13), 4968-4973.

- Fries, I. (2010). *Nosema ceranae* in European honey bees (*Apis mellifera*). *Journal of Invertebrate Pathology* 103(SUPPL. 1).
- Gregorc, A., and Ellis, J. D. (2011). Cell death localization in situ in laboratory reared honey bee (*Apis mellifera* L.) larvae treated with pesticides. *Pesticide Biochemistry and Physiology* 99(2), 200-207.
- Gregorc, A., Evans, J. D., Scharf, M., and Ellis, J. D. (2012). Gene expression in honey bee (*Apis mellifera*) larvae exposed to pesticides and *Varroa* mites (*Varroa destructor*). *Journal of Insect Physiology* 58(8), 1042-1049.
- Harris, J. W., Danka, R. G., and Villa, J. D. (2012). Changes in infestation, cell cap condition, and reproductive status of *Varroa destructor* (Mesostigmata: Varroidae) in brood exposed to honey bees with *Varroa* sensitive hygiene. *Annals of the Entomological Society of America* 105(3), 512-518.
- Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., Aptel, J., Tchamitchian, S., and Decourtye, A. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science* 336(6079), 348-350.
- HoneybeeGenomeSequencingConsortium (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443(7114), 931-949.
- Linksvayer, T. A., Kaftanoglu, O., Akyol, E., Blatch, S., Amdam, G. V., and Page, R. E. (2011). Larval and nurse worker control of developmental plasticity and the evolution of honey bee queen-worker dimorphism. *Journal of Evolutionary Biology* 24(9), 1939-1948.
- Nazzi, F., Brown, S. P., Annoscia, D., Del Piccolo, F., Di Prisco, G., Varricchio, P., Vedova, G. D., Cattonaro, F., Caprio, E., and Pennacchio, F. (2012). Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathogens* 8(6).
- Oxley, P. R., Spivak, M., and Oldroyd, B. P. (2010). Six quantitative trait loci influence task thresholds for hygienic behaviour in honeybees (*Apis mellifera*). *Molecular Ecology* 19(7), 1452-1461.
- Page Jr, R. E., Fondrk, M. K., and Rueppell, O. (2012). Complex pleiotropy characterizes the pollen hoarding syndrome in honey bees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 66(11), 1459-1466.

- Pettis, J. S., Vanengelsdorp, D., Johnson, J., and Dively, G. (2012). Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. *Naturwissenschaften* 99(2), 153-158.
- Rueppell, O., Hayworth, M. K., and Ross, N. P. (2010). Altruistic self-removal of health-compromised honey bee workers from their hive. *Journal of Evolutionary Biology* 23(7), 1538-1546.
- Runckel, C., Flenniken, M. L., Engel, J. C., Ruby, J. G., Ganem, D., Andino, R., and DeRisi, J. L. (2011). Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, Nosema, and Crithidia. *PLoS ONE* 6(6): e20656.
- Siede, R., Meixner, M. D., and Büchler, R. (2012). Comparison of transcriptional changes of immune genes to experimental challenge in the honey bee (*Apis mellifera*). *Journal of Apicultural Research* 51(4), 320-328.
- Singh, R., Levitt, A. L., Rajotte, E. G., Holmes, E. C., Ostiguy, N., Vanengelsdorp, D., Lipkin, W. I., Depamphilis, C. W., Toth, A. L., and Cox-Foster, D. L. (2010). RNA viruses in hymenopteran pollinators: Evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* hymenopteran species. *PLoS ONE* 5(12).
- Williams, G. R., Dietemann, V., Ellis, J. D., and Neumann, P. (2012). An update on the COLOSS network and the BEEBOOK: Standard methodologies for *Apis mellifera* research. *Journal of Apicultural Research* 51(2), 151-153.

## Appendix 5. Cited References for Pesticide Research Presentation

- Ciarlo, T. J., C. A. Mullin, J. L. Frazier and D. R. Schmehl. 2012. Learning impairment in honey bees caused by agricultural spray adjuvants. *PLoS ONE*, 7, e40848-e40848.
- Cresswell, J. E. and H. M. Thompson. 2012. 'Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"', *Science* 337, 1453.
- Eiri, D and J.C. Nieh. 2012. A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing. *J Exp Biol.* 215:2022-2029.
- Hawthorne DJ, Dively GP. 2011. Killing them with kindness? In-hive medications may inhibit xenobiotic efflux transporters and endanger honey bees. *PLoS One.* 2011; 6 (11):e26796.
- Johnson RM, Mao W, Pollock HS, Niu G, Schuler MA, et al. 2012. Ecologically Appropriate Xenobiotics Induce Cytochrome P450s in *Apis mellifera*. *PLoS ONE* 7(2): e31051.
- Johnson RM, Dahlgren L, Siegfried BD, Ellis MD (2013) Acaricide, Fungicide and Drug Interactions in Honey Bees (*Apis mellifera*). *PLoS ONE* 8(1): e54092.
- Khoury DS, Myerscough MR, Barron AB (2011) A Quantitative Model of Honey Bee Colony Population Dynamics. *PLoS ONE* 6(4): e18491. doi:10.1371/journal.pone.0018491
- Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields. *PLoS ONE* 7(1): e29268. doi:10.1371/journal.pone.0029268
- Mullin, C.A., M. Frazier, J. L. Frazier, S. Ashcraft<sup>1</sup>, R. Simonds, D. vanEngelsdorp, J. S. Pettis. 2010. High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. *PLoS. ONE*.5 (3): e9754.
- J. Pistorius, G. Bischoff, U. Heimbach, M. Stähler. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize, In: Oomen and Thompson (Eds). *Hazards of pesticides to bees: 10th Int. Symp. of the ICP-BR Bee Protection Group, Bucharest (Romania), Oct 8-10, 2008.* pp118
- Schneider C. W., Tautz J., Grünewald B., Fuchs S. (2012). RFID tracking of sub-lethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS ONE* 7, e30023.

Teeters, B.S. R. M. Johnson, M D. Ellis, B D. Siegfried. 2012. Using video-tracking to assess sublethal effects of pesticides on honey bees (*Apis mellifera* L.), Environmental Toxicology and Chemistry. 1349–1354

## **Appendix 6. Cited References for Biology, Genetics, Breeding Research Presentation**

- Delaney, D. A., M. D. Meixner, N. M. Schiff, and W. S. Sheppard. 2009. Genetic Characterization of Commercial Honey Bee (Hymenoptera: Apidae) Populations in the United States by Using Mitochondrial and Microsatellite Markers, *Annals of the Entomological Society of America*, 102(4):666-673.
- Harpur, B.A., S. Minaei, C.F. Kent and A. Zayed. 2012. Management increases genetic diversity of honey bees via admixture, *Molecular Ecology* 21, 4414–4421.
- Sheppard, W. S. 1989. A History of the Introduction of Honey Bee Races Into the United States: Parts I and II of a Two-part Series, USDAARS, Beneficial Insects Laboratory, Bldg. 476, BARC-East Beltsville, Maryland 20705.

**TAB 4**



and collected brood measurement data in response to different diets under Dr. Standifer's supervision. I also worked collecting data on onion seed pollination studies, under Dr. Waller's direction, which were used to analyze honeybee visitation habits and preferences. Under Senior Scientist Steve Taber's supervision, I raised queen bees for his queen breeding experiments and additional queen bees for use in other studies at the Laboratory. Mr. Taber exposed me to his hygienic behavior study and hypothesis, which at that time was disputed by academia, but is now full accepted.

3. I am a member of the American Honey Producers Association, the California State Beekeepers Association, Sue Bee Honey Association, and the Yuma County Farm Bureau. I serve on the Board of Governors of the Yuma County Water Users Association.

4. I was raised in a commercial beekeeping and farming family business. My father owned a 10,000+ colony beekeeping operation and was a farmer of up to 500 acres. By the time I arrived at the Tucson Bee Laboratory, I had acquired the skills of a commercial beekeeper, and had raised queens on a commercial level for many years. I began work as an independent commercial beekeeper after graduation with a 1,000 colony business.

5. In 1997, I was asked by my family to form and manage James R. Smith Beekeeping and Farming, LLC due to the death of my father. Today, I hold

the controlling interest in that business. We own and directly lease approximately 500 irrigated acres in Yuma County, Arizona, where we grow cotton, Sudan grass, and wheat. We also presently operate about 3,850 honeybee colonies. In addition to me, the business employs nine full-time workers.

6. Our business provides pollination services to farmers in Arizona for pollination of a wide variety of vegetable seed crops, such as broccoli, cauliflower, onions, artichokes, Chinese cabbage, and cilantro. We also provide pollination for cantaloupe, watermelon, alfalfa seed, and hybrid canola seed. In addition, we lease about 800 to 1000 of our hives each winter to a business that provides pollination services to almond growers in California.

7. Our beekeeping business has suffered extensive losses in recent years. Prior to 2008 we managed 10,000 to 14,000 colonies. At two different times prior to 2008, I chose to reduce the number of colonies and sold a total of approximately 6,500 colonies. In 2007, I sold 4,600 colonies thereby downsizing the business to about 7,000 colonies. Then, in January 2008, 6,000 of 7,280 hives – over 80% of our honeybee business – failed over the course of just 45 days. The death of those colonies represented a 2008 income loss of \$720,000 and a 2008 equity replacement loss of \$540,000 for the live bees. Eight long-time employees had to secure other employment. It was the hardest decision I ever had to make. Those

men were the core to the success of the business. They had to accept lower paying jobs in new fields of work to support their families.

8. Since January 2008, I have continued to experience difficulty in maintaining colonies. No longer can I increase numbers as in prior years. The amount of inputs, in the form of dietary supplements and labor, which are required to maintain colony numbers have risen dramatically. Since 2008, it is no longer possible for me to maintain the consistent colony strength required for pollination without those costly dietary supplements, labor, and transportation inputs.

9. My commercial beekeeping business earns about 80% of its income from the pollination of crops and 20% from honey production. Many of the crops which my bees pollinate, and other crops grown in the area which are used as forage, may be sprayed with sulfoxaflor. Some of those crops are permitted to receive up to four applications of sulfoxaflor per growing season, which will result in multiple exposures – and sulfoxaflor stays in and on the plant for a number of days.

10. According to the official Arizona Department of Agriculture's pesticide registration website, sulfoxaflor has been registered for use in Arizona, and it is my understanding from neighbors, formal notifications I receive, and from registration information that sulfoxaflor is already being applied on crops – including cotton – near Yuma where my bees forage and where I provide

commercial pollination services. Cotton is a major source of summer nectar for my bees.

11. While I try to take protective action, it is very difficult due to the widespread use of sulfoxaflor and pesticides like it. For example, while I may sometimes receive notice of pesticide applications, the notice is usually short and would require me to act overnight. Moreover, moving my bees to protect them from a pesticide application at one location would only put them at risk to another application at the new location – there is simply nowhere “safe” to put them. When I have, for example, 3,000-4,000 bees within flight range of an area of 1,000 acres that someone has notified me will be sprayed, it is impossible to move in one night. Instead, it is more likely that I will have to take additional steps to feed supplements and spend time and resources splitting and caring for hives that are suffering in order to keep my entire operation from crashing similar to what happened to me in 2008. Therefore, not only has my income from bee-keeping diminished, I will likely have even higher costs to provide minimal protections as a result of this new exposure to this highly-toxic pesticide.

12. I have reviewed the ecological risk assessment prepared by the Environmental Protection Agency (“EPA”) for sulfoxaflor. My evaluation of the toxicity studies described in the risk assessment in conjunction with the resulting pesticide labels leads me to conclude that the registration of sulfoxaflor will result

in unacceptable, continuing, increasing damage to managed honeybee colonies, and other pollinators that are already suffering the cumulative effects of pesticide exposure on our national landscape. Among other shortcomings, the field studies evaluated by EPA are grossly inadequate to assess the impact that exposure to sulfoxaflor will have on bees, and the colony organism.

13. EPA also did a very one-sided and constrained analysis of “benefits,” looking only at pesticide-use benefits, but not at the critical benefits of bees to agricultural crops which require insect pollination, or the economic losses that pesticides cause to beekeepers and agriculture businesses that are dependent on pollination. I summarize EPA’s value of the pollinator as virtually nonexistent unless a crop, such as cotton, soybeans, or canola receives a substantial direct yield benefit and that the yield is reliant on pollinators.

14. I am seeking reconsideration of EPA’s decision to register sulfoxaflor because EPA failed to consider important factors that I believe must be considered when making a decision to register a pesticide and when deciding how to control its use. If EPA reconsiders and properly analyzes these things, I believe that it will come to a decision that is more protective of bees.

15. The “advisory pollinator statements” that EPA established for some crops that continually flower, including cucurbits and citrus, will not prevent harm to bees. I have observed bees foraging in the early morning twilight hours before

sunrise, which occurred at 5:31 AM on June 1, 2013 in Yuma. I have also observed honey bees foraging until complete darkness, which occurred at 7:42 PM on that same date. Also, tests have shown that sulfoxaflor residue remains acutely toxic to bees for at least four hours after the pesticide has dried, meaning that an application near 7:00 AM will still be directly toxic to my bees at 10:00 AM when they are in full forage mode, to say nothing of systemic effects. Moreover, the advisory language regarding timing of applications is not even mandatory; it is simply an “advisory,” lacking mandatory language which would actually protect bees from direct lethal contact with sulfoxaflor or from the transport of contaminated pollen or nectar during the days immediately following a pesticide application. Finally, the advisory language regarding timing of applications does not apply at all to cotton – one of the most prevalent and significant continually-flowering crops.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge and belief.

Dated: October 16<sup>th</sup>, 2013

  
Thomas R. Smith

**TAB 5**



3. AHPA is a non-profit agricultural association incorporated in Oklahoma in 1969. The organization is dedicated to promoting the common interest and general welfare of the American honey producer. AHPA currently has about 400 members who make their living from the production of honey. Collectively, I estimate that AHPA members produce as much as 50% of the United States' honey.

4. I have been a commercial beekeeper since 1990, when an opportunity arose for my wife and me to purchase 750 beehives from her grandfather and to be mentored by his 60 years of beekeeping experience. Our business, Harvest Honey, Inc., grew significantly over the years, and we now run about 18,000 hives.

5. Harvest Honey is based in Bismarck, North Dakota. We run our bees in North Dakota from about April to October each year, usually in close proximity to fields of corn, soybeans, sunflowers and canola. Once honey production winds down in late summer, we haul our bees to southern Texas near Houston, where there is usually abundant winter forage. Around the new year, we take our bees to the central valley of California, where we contract to provide pollination services for the almond bloom. Upon completion of the almond pollination season, the bees are shipped to back to Texas to make up summer and winter losses, before eventually returning to North Dakota.

6. While it is not uncommon to have some hive losses over the course of a year in beekeeping, at Harvest Honey our annual hive losses have increased to as much as 50% per year since the mid-2000s. Our experience is not unique. Preliminary survey results from the U.S. Department of Agriculture suggests that 31% of managed honey bee colonies in the United States were lost during the 2012 to 2013 winter. This represents an increase in loss of 42% over the previous 2011/2012 winter's total losses. The 2012/2013 winter losses are on par with the 6 year average total loss of 30%. *See* <http://beeinformed.org/2013/05/winter-loss-survey-2012-2013/>.

7. The dramatic increase in hive loss in recent years has had a huge impact on the beekeeping and pollination business. I know beekeepers that have been forced out business because their annual losses became unsustainable – they simply could not replace lost bees with healthy bees fast enough. Others are hanging on, hoping things get better but facing economic struggles every year. My own business is much less profitable than it was, due in part to the added costs of replacing hives that are lost each year and the added costs of feeding weakened colonies food supplements.

8. If colony losses continue at or above the 30% level, it will threaten the economic viability of the bee pollination industry. The cost of honey bee

pollination services will rise significantly, and those increased costs will ultimately be passed on to consumers through higher food costs.

9. The decision of the Environmental Protection Agency (“EPA”) to register yet another pesticide that is systemic and highly toxic to bees poses a direct and immediate threat to my business. As part of keeping up on developments that affect my industry and my work with AHPA, I understand that neonicotinoid pesticides gained prevalence in the mid-2000s and that their use has increased every year since. I also know that research has pointed to neonicotinoid pesticides as at least part of the cause for the bee crisis we are currently facing. Sulfoxaflor is an additional pesticide threat on top of what is out there already.

10. According to the North Dakota state pesticide registration website, sulfoxaflor has been registered for use in North Dakota for soybeans and some additional crops. My bees forage in and near soybean fields as they are a dominant crop in many parts of North Dakota. Sulfoxaflor has also been registered for use in Texas, according to the National Pesticide Information Retrieval System website.

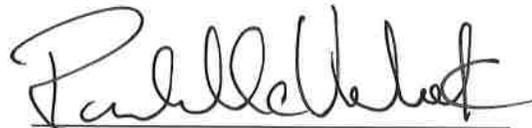
11. My bees risk exposure to sulfoxaflor not only when the pesticide is applied, but also when foraging on crops that have been sprayed with sulfoxaflor or on other flowering weeds in or near the pesticide-treated crops. Forager bees will carry the sulfoxaflor-tainted pollen and nectar back to the hive, where it will

be consumed by the developing brood. In this way, sulfoxaflor impacts the entire hive.

12. In my opinion, EPA did not properly consider many of the issues for the beekeeping and pollination industry when it registered sulfoxaflor. AHPA has asked EPA to reconsider its decision so as to be more protective of bees, either by denying registration or by including more protections for bees and the economics of the beekeeping industry. We are a critical part of agriculture in this country, and I think EPA failed to take that into proper consideration.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge and belief.

Dated: October 7, 2013



Randell C. Verhoek