

# **SUPERBOOST: A SCIENTIFIC SUMMARY**

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## **CAPSULE DESCRIPTION OF THE PRODUCT AND ITS USES**

- SuperBoost is a blend of 10 non-volatile fatty acid esters that precisely reproduces the composition of the natural honey bee brood pheromone. It has the consistency of vegetable oil.
- In nature, honey bee larvae produce the pheromone, which tells worker bees “we’re here, and we’re hungry”. In turn, the workers forage with increased intensity for pollen and nectar. Nutrition of the larvae and the queen improves, the queen lays more eggs, and colony vigor is enhanced.
- SuperBoost differs in composition from the natural pheromone only in that a small amount of a food-grade antioxidant is added to prevent rapid breakdown of four very unstable pheromone components.
- SuperBoost is administered to a colony in a small plastic pouch containing 180 mg of pheromone. The pouch is held in a retrievable plastic holder that suspends the pouch between two frames at the level of the brood comb. The synthetic pheromone exudes through the plastic membrane of one side of the pouch in milligram (or sub-milligram) amounts per day over a five-week treatment period. Worker bees pick up the pheromone by contact with the surface of the membrane.
- Treatment of a honey bee colony with SuperBoost amplifies the effect of the natural pheromone that is already present, telling the colony that there are more larvae present than there really are.
- Experimental research has shown that treatment with SuperBoost increases the number of foraging trips, increases the visitation rate to flowers, causes more pollen to be brought back to the hive, increases brood comb area and the size of the adult work force, substantially increases the honey harvest, improves consumption of pollen substitute fed to colonies in the fall and at the end of winter, enhances survival of colonies over the winter, and enables a beekeeper to split vigorous colonies more frequently to produce additional colonies.

## **NATURAL OCCURRENCE AND DEMONSTRATED EFFECTS OF BROOD PHEROMONE**

The honey bee brood pheromone, is a non-volatile blend of methyl and ethyl esters of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid (LeConte et al. 1990). It has the consistency of vegetable oil. It is produced in the salivary glands of larval honey bees (LeConte et al. 2006). On average, each worker larva contains 560 ng of pheromone (Pankiw 2004). However, because pheromone is produced and released constantly, a larva will release much more than 560 ng before it pupates. In contrast to worker larvae, the total pheromone content of a drone larva (which is 2.5 times heavier than a worker larva) at the time the larval cell is capped reaches a peak of 1,850 ng (Trouiller et al. 1992).

Brood pheromone has been shown to have both primer (physiological and biochemical) and releaser (behavioral) effects (LeConte et al. 2006).

Two notable primer effects are:

- dose-dependent lowering or raising the age at which workers first forage outside the hive (LeConte et al. 2001; Pankiw et al. 2004; Sagili et al. 2011); and
- stimulation of the hypopharyngeal and mandibular glands to produce enriched protein (royal jelly) that is fed to larvae and the queen, in turn increasing larval vigor, and enabling the queen to produce more brood (Mohammedi et al. 1996; Pankiw et al. 2004; Sagili and Pankiw 2009; Peters et al. 2010).

Demonstrated behavioral effects of synthetic brood pheromone (not SuperBoost) administered to honey bee colonies include:

- increased ratio of pollen to non-pollen foragers (Pankiw and Page 2001; Pankiw and Rubink 2002; Lafontaine et al. 2009);
- up to 150% more pollen foragers (Pankiw 2004; Pankiw and Page 2001; Pankiw and Rubink 2002; Pankiw et al. 1998, 2004);
- increased frequency of foraging trips (Pankiw 2007);
- higher pollen loads returned by foragers (Pankiw 2004; Pankiw et al. 2004);
- greater number and higher activity of non-pollen foragers, which (assuming that they are nectar foragers) would further enhance pollination (Pankiw 2004; Pankiw et al. 2004); and
- increased consumption of dietary pollen substitute at the end of winter, leading to increased colony vigor (Pankiw et al. 2008, 2010).

## **PRODUCT DEVELOPMENT AND DESCRIPTION**

Despite the plethora of potentially beneficial effects of brood pheromone treatment, development of a commercial product was delayed by two impediments. The first was a very limited shelf life for the synthetic pheromone, because four of the 10 components are unstable and easily oxidized (Page and Pankiw 2003). The second challenge was to develop a device that gradually released satisfactory amounts of the pheromone over a sufficiently long duration to produce a positive effect.

The first problem was solved by developing a proprietary formulation that incorporated a food-grade antioxidant, tertiary butylhydroquinone, into the pheromone blend. The percentage composition by weight of the components in the blend is: methyl palmitate 2.11%, ethyl palmitate 4.19%; methyl stearate 17.30%, ethyl stearate 8.04%, methyl oleate 21.66%, ethyl oleate 7.50%, methyl linoleate 8.09%, ethyl linoleate 3.82%, methyl linolenate 16.53%, ethyl linoenate 10.77% and tertiary-butylhydroquinone 0.05%. This formulation remained stable over a two-year test period (Pankiw et al. 2011). There is evidence that the ratio of components differs among honey bee races, and that bees respond optimally to the ratio produced within each race (Metz et al. 2010).

Two more years were required before a suitable release device was developed. This is small plastic pouch with a pheromone-permeable polyethylene membrane, a Mylar backing, and a capacity of 180 mg (200  $\mu$ L). In the laboratory the pouch released 0.3 mg of stabilized synthetic brood pheromone per day

for 10 days, when the exuded pheromone was removed by swabbing the exterior surface (Pankiw et al. 2011).

A prototype SuperBoost device, consisting of the pouch mounted in a 35 mm plastic slide frame suspended by a wire between frames at the level of the brood comb, was tested in September-October 2007 in southeast Texas (Pankiw et al. 2011). Since 2007, Contech has continued to refine this product. We determined that manufactured pouches must be stored frozen to prevent deterioration of the pheromone release membrane. We also developed a new holder that prevents the pheromone release membrane of the pouch from resting against the comb, where bees would not be able to contact the exuded pheromone (M. Foster et al. 2011).

## **OPERATIONAL EFFICACY OF SUPERBOOST IN THE FIELD**

To date, the following positive effects of SuperBoost have been experimentally demonstrated in the field:

- higher ratios of pollen to non-pollen foragers in SuperBoost-treated than control colonies for over five weeks in September-October in southeast Texas, 2007 (Pankiw et al. 2011);
- heavier pollen loads on workers returning to SuperBoost-treated colonies than on returning workers from untreated control colonies, September-October in southeast Texas, 2007 (Pankiw et al. 2011);
- 47% increase in brood comb area compared to control colonies, which declined by 24% after five weeks, September-October in southeast Texas, 2007 (Pankiw et al.,2011);
- 36% increase in adult population size compared to control colonies, which declined in size by 10.7% after five weeks, September-October in southeast Texas, 2007 (Pankiw et al. 2011);
- 50% higher consumption of pollen substitute diet fed to overwintered colonies than by control colonies after 10 weeks of SuperBoost treatment, January-April in the lower Fraser Valley of British Columbia (Moeri et al. 2011; Lait et al. 2012);
- 2.4 times greater area of brood comb in SuperBoost-treated overwintered colonies than in control colonies, January-April in the lower Fraser Valley of British Columbia (Moeri et al. 2011);
- double the adult population level after 10 weeks of SuperBoost treatment at the end of winter compared to control colonies, January-April in the lower Fraser Valley of British Columbia (Moeri et al. 2011);
- 3.3 times more SuperBoost-treated colonies producing splits during spring build-up than control colonies, January-April in the lower Fraser Valley of British Columbia (Moeri et al. 2011);
- 3 times more SuperBoost-treated colonies producing splits during spring build-up than control colonies, September-October on the north island, New Zealand (equivalent to March-April in the northern hemisphere) (B. Foster et al. 2011);
- 68.5% and 16.6% greater pollen substitute diet consumption in the fall feeding period by SuperBoost-treated colonies compared to control colonies in Salem and Hermiston, Oregon, respectively (Sagili and Breece 2012);

- 37.1% and 38.3% greater brood comb area in colonies treated with SuperBoost in the fall feeding period compared to control colonies in Salem and Hermiston, Oregon, respectively (Sagili and Breece 2012);
- 32.3% and 40.5% more adult worker bees in colonies treated with SuperBoost in the fall feeding period than in control colonies in Salem and Hermiston, Oregon, respectively (Sagili and Breece 2012);
- 75.6% more honey frames and 84.3% more honey harvested from package bee colonies treated for 15 weeks with SuperBoost compared to control colonies, April-August, Lower Fraser Valley of British Columbia (Lait et al. 2012);
- 78% more honey harvested from established colonies treated for 10 weeks with SuperBoost compared to control colonies, April-September, Lower Fraser Valley of British Columbia (unpub. data);
- 23% more honey harvested from established colonies treated for 10 weeks with SuperBoost compared to control colonies, September 2010-March 2011 on the north island, New Zealand (equivalent to March-September in the northern hemisphere) (B. Foster et al. 2011);
- 2.6 times greater survival of package bee colonies treated with SuperBoost in spring 2009, fall 2009 and spring 2010 than for control colonies, Lower Fraser Valley of British Columbia (Lait et al. 2012);
- more splits from package bee colonies treated with SuperBoost in spring 2009, fall 2009 and spring 2010 than from control colonies, Lower Fraser Valley of British Columbia (Lait et al. 2012);
- 40% higher visitation of flowers in seed carrot fields pollinated by SuperBoost-treated colonies than by control colonies, Willamette Valley, Oregon (Sagili 2010);
- in six replicates, 26% more carrot seed harvested from fields pollinated by SuperBoost-treated colonies than from fields pollinated by untreated control colonies, Willamette Valley, Oregon (in collaboration with Central Oregon Seeds Inc.) (Sagili 2012).

## **SAFETY TO HONEY BEES**

There has been some concern among beekeepers that treatment with SuperBoost could cause undue stress to overworked workers and queens. However, in all studies conducted to date (including basic studies with synthetic brood pheromone offered daily to colonies on glass plates suspended between the frames in a hive), there have been no negative effects observed. Most of these have been short-term studies, e.g. 10 weeks of continuous treatment coupled with pollen substitute feeding at the end of winter, with observations ending at 96 days (Moeri et al. 2011). In this study SuperBoost-treated colonies consumed more pollen substitute, had more brood comb area, had higher adult populations and produced more daughter colonies (splits) than untreated controls.

A long-duration study was conducted by Lait et al. (2012) with colonies established on 30 April 2009 in British Columbia from 1.3 kg (3 lb) packages of bees imported from New Zealand. Half of these colonies were treated with SuperBoost immediately, with treatment lasting 15 weeks. They were treated again for five weeks in the fall during the pre-winter period of feeding with pollen substitute, and again for seven weeks during the spring build-up pollen substitute feeding period. Treated colonies produced

significantly more honey than control colonies, and entered the fall feeding period with 35.4% more adults than control colonies. By 21 April 2010, some of the original treated colonies had died over the winter, but these were replaced by splits from vigorous colonies, resulting in 80.0% of the original number of colonies being alive and vigorous. In contrast, only 31.4% of the untreated control colonies survived the winter, and these produced almost no daughter colonies. The surviving SuperBoost-treated colonies were tracked for an additional summer (2010), and there was no indication of any adverse effect.

It should be noted that (except for the stabilizing antioxidant) treatment with SuperBoost does not introduce anything that is not already present in the hive. Rather, it is a supplement to the natural pheromone that is already being produced by each larva in the colony, and conveys the message to foragers that there are more larvae to feed than are actually present in the colony.

If the amount of brood pheromone released averaged 1.6 mg per day (the highest rate observed in research trials), and if a single larva contains 560 ng of pheromone (Pankiw 2004), the daily dose would be equivalent to the pheromone produced by 2,857 larvae. For comparison, a small colony of 5,000 bees can be expected to have 5,000 larvae (Hoopingarner and Waller 1992). As the colony grows in size the ratio of bees to larvae changes gradually in favor of bees, so a colony of 50,000 bees can be expected to have approximately 17,000 larvae.

## **SAFETY TO HUMANS**

All of the components of SuperBoost (except for the antioxidant) are produced in nature by honey bee larvae. The most likely means of exposure to any of the components of SuperBoost, or to the natural pheromone present in the hive, would be through the dietary consumption of honey. However, examination of the available information leads to the conclusion that there is absolutely no cause for concern about potential adverse effects on human health caused by treating honey bee colonies with SuperBoost. The evaluation includes the following information:

- all of the fatty acids and their esters in the honey bee brood pheromone (and thus in SuperBoost) are found naturally as trace constituents in honey (Smith and McGaughey 1966; Kapoulas et al. 1977; Tan et al. 1988, 1989, 1990);
- all of the fatty acids that appear as methyl and ethyl esters in the honey bee brood pheromone (and SuperBoost) are present in large amounts in many natural materials, including the lipid of honey bee larvae and adults (Manning 2001), the pollen of many plants pollinated by honey bees, such as dandelions (Standifer 1966), rapeseed (canola) (Evans et al. 1987) and almonds (Loper et al. 1980), and many foods consumed daily by humans, including butter, corn, peanuts and soybeans ([www.welch-holme-clark.com](http://www.welch-holme-clark.com)).
- as early as 1932, two of the components of the honey bee brood pheromone (and SuperBoost), methyl linoleate and methyl linolenate, were shown to have therapeutic value in the diet, restoring lipid-deficient rats to good health (Burr et al. 1932);
- honey contains 17.2% water; only two of the components of SuperBoost are soluble in water (ethyl stearate at 0.0039 mg per L and ethyl palmitate at 0.0037 mg per L) (Stecher 1960), making it improbable that any component would be taken up readily in honey;
- the most probable way that ethyl stearate and ethyl palmitate, as well as other components of brood pheromone (and SuperBoost), could appear as trace components of honey would be as solutes in the very small amount of beeswax found naturally in honey;

- if a honey bee colony produced a very conservative 10 kg of honey (most produce 20 kg or more), and in the improbable event that ALL of the 180 mg of SuperBoost in a single treatment ended up in the honey, the concentration in the honey would be only 18 ppm;
- despite the relatively large amount of synthetic brood pheromone that is present in a single SuperBoost device, the actual amounts released per day into a colony range from approximately 0.4 – 1.6 mg per day (Sagili 2009; Moeri et al. 2011); and
- the tertiary-butylhydroquinone that is added at 0.05% to stabilize the SuperBoost composition is a commonly used food preservative for unsaturated vegetable oils and animal fats, with a high limit of 1,000 mg per kg permitted for frozen fish and fish products (<http://en.wikipedia.org/wiki/Tert-Butylhydroquinone>).

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