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Honeybee visitors to cotton flowers and their role in crop pollination

A Literature Review

Saul A. Cunningham
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Executive Summary

This literature review gathered evidence regarding: (1) the potential for honeybee pollination to significantly contribute to cotton yield in Australia and (2) the attractiveness of cotton crops to foraging honeybees. Thirty one studies report effects of bee pollination on cotton yield and all indicate benefits, but they range widely in the quality of the evidence they provide. Many studies are not supported by full English language reports and others show benefits in varieties or planting systems that are not representative of modern Australian practice. Critically, most studies quantify the effect of bee pollination by contrast to total pollinator exclusion, whereas cotton growers need to know the yield benefit that would be achieved by adding managed honeybees to an open pollinated field. Therefore, although the evidence is strong that bee pollination can play an important role, new experiments are required to determine if there would be an economic benefit to Australian cotton growers from using managed honeybees. Studies of honeybee foraging indicate that cotton is not a highly attractive crop and there can be period of low visitation as a result. Nevertheless, there are also studies showing periods of high visitation including both nectar and pollen gathering. This high variation in apparent attractiveness is consistent with the expectation that honeybees forage over a large area and will make choices among a range of different resources in the environment, which vary over time. Cotton flowers are attractive enough that honeybees are usually reported as the most common flower visitors, and in many studies they visit frequently enough to be effective pollinators.

Background and scope

Interactions between honeybees and cotton flowers are important to the cotton industry for two reasons. Firstly, it may be that cotton growers could benefit from honeybees because of their potential role in crop pollination. Secondly, growers need to understand the way in which honeybees use the cotton crop environment so that they can ensure that their farm management does not create unnecessary risks for bees. Chief among the risks is that application of insecticide while bees are foraging can kill bees. Management of cotton production could therefore be improved by understanding the nature of interactions between honeybees and the cotton crop. This report presents a literature review that is designed to help achieve this objective. The literature review focuses on evidence relevant to the following questions:

(1) Can honeybee pollination significantly contribute to cotton yield (lint or seed)? Is there potential to increase yield through better pollination outcomes?
(2) How attractive are cotton flowers to bees? Can we assess how likely it is that bees will be drawn into these crops?

The review also describes the knowledge gaps regarding these questions and suggests what research activities could address the gaps. The review does not focus on questions regarding the relative toxicity of different pesticides or hive management strategies to reduce the risk of bee kills.

Pollination as a determinant of yield

Pollination is the process by which pollen is moved from anthers to the stigma. In most plants this is a necessary precursor to fertilization, whereby the sperm cell is united with an egg cell in the ovary to initiate a seed. For almost all seed and fruit crops pollination is an essential step in process of production (the exception being parthenocarpous crops, like bananas, that have been bred to create fruits without seeds). For many crops pollination is not a limiting process, either because wind moves pollen freely, or because self pollen (such as will move easily within a flower) is sufficient to maximise the yield. But for other crops pollen transfer requires animal pollinators (especially insects) and pollen movement between plants (rather than within flower) is necessary or at least advantageous. The degree to which pollination limits yield also depends on the balance with the supply of other resources. If, for example, soil fertility is strongly limiting seed production, then increasing the pollination rate may not lift yield. In summary, pollination is a critical process in producing seeds, but whether or not it is an important consideration in farm practice depends on the plant's mating system (i.e. the relative importance of outcrossing), mode of pollination (i.e. wind, insects, or other) and the capacity for plants to respond if more seeds are fertilised (i.e. other limits to seed development).

There is increasing evidence that crop pollination by insects plays a bigger role in constraining yield than had been formerly recognised (Klein et al 2007, Garibaldi et al 2013). Part of this re-assessment reflects a slowly growing recognition that even self-fertile crops can benefit from increased pollination, because the genetic advantage of outcrossing leads to increased quality and number of seeds. There is also a view that pollination shortages may be revealing themselves more frequently because of a general decline in the abundance of wild pollinators in our agricultural landscapes (Garibaldi et al 2011). Of course, agronomic practices also change, as do the varieties (and therefore the genetic composition) of crops being planted, so that the relative importance of crop pollination is constantly shifting. From a grower's point of view, however, the important question is "could I produce a more profitable crop by paying more attention to insect pollination?" The answer to this question requires consideration of the breeding system of the plant, the relative role of insect pollinators, and the capacity to integrate any new practices (e.g. managed pollination by honeybees) into the agronomic system.

Here I examine the evidence that cotton production could be improved by use of managed honeybees. Keshlaf (2008) provided a quite detailed summary of the literature on this topic in the introduction to his thesis. The goal of this review is not to repeat all the information presented there, but to focus on the *quality* and *relevance* of the data available to assess pollination benefits to cotton. It should be noted, however, that in the Keshlaf review pollination studies were cited as evidence as of a pollination benefit to cotton even when they were conducted on male sterile hybrid seed trials, which (argued below) is not particularly relevant for most cotton growers. In this review I put a strong emphasis on identifying which studies are most relevant to the question “could providing managed honeybees lift yield for cotton grown under typical circumstances in Australia?”

Literature search

To collect references for this literature review I used a number of different methods. First, I used the “Web of Science” database (http://thomsonreuters.com/thomson-reuters-web-of-science/) and searched on the terms “cotton, pollin*, bee”, which returned 244 items. Second, I used Google Scholar (http://scholar.google.com.au/), searching the phrase “cotton pollination bee” and searched through more than 10 pages of records until the quality of the match declined to a point that new records were not useful. Finally I uncovered new references from the bibliography pages of the main references, and from my own database of pollination literature established over two decades of research. Abstracts and titles were used to determine if studies were relevant to the focal questions of this review. The search was designed to uncover all of the most relevant studies, but the cotton pollination literature is even larger if one includes studies of pollinators in male sterile seed production systems, studies of gene flow (particularly relevant for GM systems) and studies of pesticide toxicity for pollinators. I gave these studies a low priority, because they generally provide little that is relevant to the core questions of the literature review (see scope), but have listed these papers in Appendix A as an additional resource.

Methods of inquiry

Different studies have applied different methods to contribute to our understanding of the role of insect pollinators in cotton production. In this review of the literature the goal is to collect the evidence from these studies and to critically review the degree to which the studies provide evidence that pollination can be improved by changes in practice. Before assessing the literature it is useful to consider the hierarchy of information that could contribute, here organised from least to most to direct evidence for a benefit from managed honeybees:

(1) *Observation of insect visitors to flowers*. This can be useful in establishing the identity and behaviour of potential pollinators, but is not informative with regards benefits to the plant in terms of seed set, lint production or quality traits.
(2) *Hand pollination of flowers*. This technique can be used to compare the relative rate of fertilisation from self vs. outcross pollen and therefore helps determine the inherent mating system for the cotton variety in question. If hand pollination is compared to a “no pollination” treatment (e.g. flowers inside an exclusion bag) the comparison indicates the relative importance of pollination as a process, but does not indicate whether it is limiting in normal field conditions (usually referred to as “open pollination”). If hand pollination is used to *supplement* natural levels of pollination, and then compared to an open pollination control, then the result indicates the potential to raise pollination above the level experienced in that trial (i.e. above “open pollination”), but does not indicate whether managed honey bees can achieve the elevated pollination rate. It is important to recognise that the baseline for comparison frames the practical question that is being addressed. Hand pollination is often applied only to individual flowers, but to link to a potential yield benefit it is

preferable to pollinate whole plants, and therefore determine whether the plant can support increased number of seeds.

(3) *Cage experiments*. Bees can be caged in a plot with plants to control bee numbers and encourage flower visiting. If caged pollination raises fruit set above open pollination then there is good evidence for the *potential* of bees to act as good pollinators, but one cannot assume that free-flying bees will behave in the same way. Cage experiments can establish a yield response on a per meter basis, rather than per plant, which is one step closer to a field scale measure. As for hand pollination, it is important to consider the baseline for comparison. Some experiments compare "caged with bees" to "caged without bees", and therefore do not provide information on the rate of pollination experienced in open conditions. Greenhouse studies can be considered a special case of the cage study, but where conditions for growth are different again from normal field conditions.

(4) *Regression studies.* If one collects data on flower visitor frequency at individual flowers, one can then examine the relationship (using regression or similar statistical models) between pollinator activity and response traits, such as probability of boll set. One way to find a gradient is to examine the effect of distance from hives. These studies document the effect of natural pollinating behaviour by bees (rather than caged bees, or hand pollinations) but because they rely on natural gradients of activity they have less experimental control.

(5) *Full field trials*. The most informative experiment (but also the most complex) is to experimentally apply managed honey bees in or adjacent to the crop, and then compare production from experimental fields and control fields with "normal" management. The best experiment links whole field yield benefit to the new pollination practice (Cunningham and Le Feuvre 2013).

Treatments and controls

The experiments described above show that different studies use different "treatments" (such as pollen addition by hand or caging with bees) and different kinds of "control" as a point of comparison (such as "pollinators excluded" or "open pollination"). Each design has different strengths and weaknesses. Regardless of the design of the experiment it is also important to know which plant variety was examined. Different varieties of the same crop type can vary greatly in their attractiveness to insect visitors, and in their mating system, so that within one crop species some varieties might be considered pollination dependant, and others not requiring pollination (Hudewenz et al 2014). This creates challenges because new varieties are developed faster than pollination biologists are doing experiments, so there is often no information on the specific variety of interest. Selection is usual focused on increasing yield, so that over time one might expect that new varieties will be less likely to suffer from yield shortfalls, such as might arise when there is a high reliance on insect pollinators. Given the knowledge gap around new varieties (regarding insect pollination experiments), it is useful to work from what is known about older varieties, but keeping in mind the possibility that new varieties are different in important ways. I have considered that studies of *G. hirsutum* varieties will be most relevant to the Australian situation. In particular, the ideal study would focus on the currently dominant Bt varieties (i.e. genetically modified *G. hirsutum*) that dominate Australian cotton plantings.

The outcome of any comparison with "open" pollination is, of course, entirely contingent on the existing level of pollinator availability in the research site. Exactly the same cultivar under the same management might be pollinator limited in a site where pollinator abundance is low, but suffering no pollination shortfall in sites where pollinators are naturally abundant. This is a particularly important issue for cotton, given that the often high level of insecticide use can have a big impact on pollinator availability. In this way the availability of unmanaged pollinators is linked to the pesticide application regime in the field. Where insecticide use is high (especially near flowering time), pollinator limitation is more likely. Where pesticide use is low and or very well targeted, there is likely to be higher availability of free pollination. So while the ideal experiment compares an experimental treatment to an open pollinated control, it needs to be recognised that the nature of the control depends strongly on the field context (e.g. are there nearby sources of feral honeybees?) and the crop management (e.g. have insecticides been applied recently?)

Finally, as with any experiments, the design and level of replication is critical if one is to have confidence in the result and the likelihood that the findings are general. The best experiments are replicated in a number of locations so that one can have confidence that the findings are relevant to a wide range of locations. But these experiments are more logistically challenging, so they are rarely conducted.

Gossypium mating system

There are many *Gossypium* species (Malvaceae), only some of which are grown commercially. Understanding the mating system variation across the genus helps set expectations for the mating system of the commercial varieties. The genus is widely regarded as self fertile, but benefiting from outcross pollination (Keshlaf 2008). The results of a pollination experiments reported in the literature (table 1) support this. However, the word outcrossing has been used in various ways by different researchers. In plant reproductive biology "outcrossing" means fertilisation by pollen from another individual plant, as distinct from self pollen. However, in agronomic literature outcrossing sometimes refers to inter-varietal cross-pollination. In crops where cultivars are produced clonally (e.g. almonds and many other tree fruits) individuals of the same cultivar are effectively clones, and therefore movement of pollen within a cultivar is very similar in genetic terms to self pollination. But with cultivars produced from seed (like cotton), individuals of the same variety are not clones, albeit that they will have been selected in a way that reduces within-variety genetic variation. For these cultivars the yield benefit from inter-varietal cross pollination might be much greater than that from within-variety outcrossing. Normal production practice in Australia is to grow large fields of one variety only, so benefits that might be associated with inter-varietal crosses are not particularly relevant to current standard practice. Studies that show higher yield from inter-varietal crosses do, nevertheless, remind us that yield is not always maximised (or resource limited) under normal circumstances, and so yield benefits from practice change are possible.

Some cotton is produced from hybrid seed, which has the good vigour often associated with hybridization (i.e. heterosis). To ensure that seed are in fact hybrid, rather than self, a male sterile line is planted to act as the seed parent and a second variety was planted to supply pollen. Pollinators are critical in this seed production system because all seed are produced by movement of pollen between plants (i.e. no selfing is possible). Indeed, many of the studies of insect pollination of cotton relate to efforts to develop effective systems for production of hybrid seed (e.g. studies 25 to 31 in table one). These studies demonstrate the base facts that bees can achieve cross-pollination in cotton, but estimates of yield benefits from these studies are not relevant to understanding the potential to improve yield in single variety fields because the circumstances are so different.

Once hybrid seed are planted, they may also respond to outcrossing in a different way to open pollinated seed. Open pollinated or fixed line varieties were developed from hybrids, but then selfed repeatedly until they are relatively homozygous. Because of these differences in origin, and the different levels of intrinsic genetic variability, it is quite likely that hybrid seed and fixed line varieties will be different in the way they respond to self and outcross pollen. For canola crops, hybrid seed planted out into the field get less benefit from pollinators than do non-hybrid varieties (pers. comm. Dr. Henrik Smith, new study in review) probably because the hybrid seed have inherently high levels of genetic diversity (e.g. heterozygosity) which reduces the genetic penalty associated with self-pollination.

Summary of literature on production benefits

All 31 studies (table 1) report bee pollination benefits to cotton production, mostly in quantity but also in some quality measures. Our search for literature on cotton pollination did not produce any studies reporting that there was no benefit to insect pollination, although it must be kept in mind that the scientific literature is generally biased toward publishing positive results and so “zero benefit” studies may be less likely to appear in the literature. The overall conclusion from this is that cotton crops have a strong potential to benefit from bee pollination. The studies were, however, conducted in a wide range of circumstances, using different cultivars in different locations and with different study designs. Therefore I have categorised each study into one of five ratings for relevance to the current Australian cotton industry. I identified 5 studies that were of “high” relevance because they examined *Gossypium hirsutum* with no intervarietal crossing. Two of these studies were conducted in Australia. I rated six studies as “potentially high” because they met the same criteria as the “high” studies, but were not available in the form of a full published report. Most of these are available only as English language abstracts from foreign language journals, and are in many cases quite old.

Ten studies report yield benefits but using study designs that allow for intervarietal crossing, or which focus on *Gossypium* species other than *hirsutum*, and are therefore only “moderate” in relevance to single variety *G. hirsutum* crops. Three studies were ambiguous in important details (Saeed et al 2012) or because they were only available in abstract form, and so were ranked as “uncertain” in their relevance.

Finally, I list seven studies that focus on the production of hybrid seed using interplanted male-sterile and male-fertile varieties. There are many more such studies in the literature, but I have listed only a few here because they have been cited by other studies as relevant to understanding cotton pollination. These have been ranked as of “low” relevance to the potential to get a yield benefit from insect pollination in a normal crop, because the use of effectively female only plants completely changes the relative importance of self and outcross pollen movement.

Table one (next page): This table summarises all the studies identified that indicate a benefit of pollination for productivity of cotton. The “relevance” column highlights the extent to which the study can be considered informative for the question “would addition of managed honeybees increase pollination in modern Australian cotton systems? And the “data quality” column note features of each study that limit the relevance or data quality. Two papers (Moffett et al 1978, MacGregor et al 1955) are split across two records each, because each reports two quite different experiments. A number of records relate to studies for which the abstract is available, but for which no full version (or full English language version) could be sourced. Two records are known only because they are referred to in other papers (Gardner and Jones 2002, Kuliev 1958). In some places quotes are used to note that text is copied from the abstract, with no further details available. If the text was ambiguous regarding, for example, the identity of pollinators, the ambiguous language was used in the table (e.g. “Honeybees”). [1]For five Russian studies the *Gossypium* species was not named explicitly in the abstract, but Ter-Avanesjan (1952) describes Russian research at a similar time, and explicitly names *Gossypium hirsutum.*

#	Authors	Year	Reported benefit from pollination	Relevance	Quality of evidence	Experimental method	Location	Species/Variety	Pollinators
1	Rhodes	2002	11.1% boll mass, 15.8% lint mass, 19.7% seed mass, 16.5% seed number. Open with bees > cage with bees > cage no bees	High	Not on Bt cotton, and bee visitation was very variable spatially.	Compare high vs. low visit plots from a distance gradient and a cage trial	Australia	Siokra V-16 cotton (*G. hirsutum)*	*Apis mellifera*
2	Keshlaf	2008	Higher boll set (51%), higher boll wt (67%), higher seed per m (80%)	High	Base line is caged plants rather than open without hives.	Caged vs. open with hives	Australia	Bt cotton (*G. hirsutum*)	*Apis mellifera*
3	Sanda et al	2013	Higher fruiting rate (36-61%), number of seeds per fruit (31-94%) and % normal seeds (33-94%) in open compared to bagged.	High	Base line is bagged exclusion and there are very large differences between years.	Bagged exclusion vs. open flowers vs. limited access	Cameroon	*Gossypium hirsutum*	*Apis mellifera adansonii*
4	Gulati et al	2005	Significantly more bolls on open plants than caged, 30% more yield in open pollinated plants with high bee visitation	High	Translation of abstract from a Japanese study so limited detail on methods	Open vs. cage, and relative visitation	India	*Gossypium hirsutum*	*Apis dorsata* and *A. florea*
5	El-Sarrag et al	1993	Comparing caged with to caged without: significantly more seed per boll (15%) seed weight (26%) yield (31%) lint per boll (23%) and oil content (12%)	High	Base line is caged plants rather than open without hives.	Caged with bees vs. caged exclusion vs. open pollination	Sudan	*G. hirsutum* Barac-B-67	Honeybees
6	Gardner & Jones	2002	25% increase in lint	Potentially high	Only a "pers comm." in Rhodes 2002, not a published reference	"Honeybee pollination"	Australia	Probably *G. hirsutum*	Honeybee
7	Trushkin	1960	“Bee pollination increases the yield by 3-5 metric cntr/ha”	Potentially high	Translation of abstract from a Russian study, so limited detail on methods	Uncertain	USSR (Samarkand)	(*G. hirsutum?*) 108-F	Bees (ambiguous)
8	Shishkin	1946	19.5% Yield benefit compared with open, 43% benefit compared with caged	Potentially high	Translation of abstract from a Russian study, so limited detail on methods	Honeybee saturation vs. open pollination vs. caged	USSR	Uncertain[1], but possibly *G. hirsutum*	Honeybees
9	Glushkov & Skrebtsov	1960	21-45% increase in yield (mass per area)	Potentially high	Translation of abstract from a Russian study, so limited detail on methods	"Control vs. saturation pollination by bees"	USSR	Uncertain[1], but possibly *G. hirsutum*	Bees (ambiguous)

10	Kaziev	1956	"Plants matured more harmoniously and 5-8 days earlier" and quality of the fibers was superior	Potentially high	Translation of abstract from a Russian study, so limited detail on methods	Uncertain	USSR (Azerbaijan)	Uncertain[1], but possibly *G. hirsutum*	Bees (ambiguous)
11	Kaziev	1960	Increase in fiber production in plants visited by bees as compared with those not visited (4-12%). Total weight of seeds increased by 10%-18%. Strength and the length of fibers increased, ripening of the pods shortened by 5-9 days. The more intensive the visitation by bees the better the yield. The increase in the crop (3-yr. average) was 19.4%-29.4%.	Potentially high	Translation of abstract from a Russian study so limited detail on methods	Uncertain	USSR	Uncertain[1], but possibly *G. hirsutum*	Bees (ambiguous)
12	Arutjunova & Skrebcov	1962	Bee pollination increased seed set, boll number, fibre yield and, to some extent, fibre quality	Moderate	Translation of abstract from a Russian study, so limited detail on methods. Appears that this experiment allowed intervarietal crossing	"Tests on the effects of intravarietal pollination"	USSR	Varieties 108F & S4727 (*G. hirsutum?*)	Bees (ambiguous)
13	Kaziev	1955	"Number of embryos was increased in the plants to which bees and insects had access: 12 and 19%. Number of ripening bolls from plants pollinated by bees was greater than from the covered plants: 12% and 22.5%. Cross pollination effected by bees improves the lint strength in all varieties	Moderate	Translation of abstract from a Russian study, so limited detail on methods. There is an indication that this experiment allowed intervarietal crossing	"Cage exclusion vs. open"	USSR (Azerbaijan)	Uncertain[1], but possibly *G. hirsutum*	Bees (ambiguous)
14	McGregor et al	1955	For Pima S-1: 25% lint mass per acre, 9% seeds per boll, 8% grams per boll lint, 10% grams per boll seed. Also less shedding of fruit. For A-44, no yield benefit but an earlier crop.	Moderate	Allowed intervarietal crossing	Caged with bees vs. caged without	Arizona, USA	Pima S-1, A-44 (*G barbadense*) and 4 vars of *G. hirsutum* (ie mixed varieties in the field)	*Apis mellifera*

15	Kaziev	1958	Seeds from plants pollinated by bees produced seedlings that appeared 3-7 days earlier and plants that grew 17-29 cm higher than those from seeds resulting from natural self-pollination. Intervarietal pollination by bees increased the number of developed bolls (23-48%) and their weight per plant (20-27%) in the 3rd generation . Fibre quality also improved.	Moderate	Translation of abstract from a Russian study: limited detail on methods. Some benefits relate to intervarietal crosses.	Uncertain	USSR (Azerbaijan)	2421, 1298, C-1472, and 2018/2 cotton varieties (*G. hirsutum?*)	Bees (ambiguous)
16	Tanda	1984	Increased boll retention by 25-51% and improved quality	Moderate	Allowed intervarietal crossing, using *G arboreum* varieties. Ambiguous whether "self" had pollinator exclusion	Open with bees provided vs. selfing	India	*Gossypium arboreum* Asiatic cotton (G27, LD133)	*Apis cerana indica*, *Apis mellifera*
17	Tanda	1983	Improved boll retention (106%), marginal effects on quality traits.	Moderate	Study species is not *G. hirsutum* (*G. arboreum)*, no significance test of boll retention effect	Open bee visited vs. tied shut (self only)	India	*Gossypium arboreum* Asiatic cotton. Variety not named.	*Apis cerana indica*, *Apis mellifera*, *Apis dorsata*
18	Wafa & Ibrahim	1960	Significant increase in bolls per plant (14%), weight of cotton seed per plant (24%), lint wt per boll (9%), wt of cotton per plot (25%) (caged with bees vs open) and large effects when comparing caged with bees to exclusion treatments	Moderate	Study species is not *G. hirsutum* (*G. barbadense*)	Total exclusion with cloth cage vs. insec exclusion vs. caged with bees vs. open, and distance gradient from hives	Egypt	Ashmouni (Egyptian cotton, *G. barbadense*)	Honeybees
19	Moffett et al	1978	Increase in number of bolls per flower (50%), number of seeds (40%) and seed wt./boll (39%)	Moderate	Intervarietal crossing allowed.	Open vs. bagged flowers, and open vs. caged. bees added to the field.	Arizona, USA	Pima S-4 (*G. barbadense*)	*Apis mellifera*
20	Moffett et al	1978	Increase in number of bolls per flower (12%), number of seeds (5%), seed wt./boll (11%) and seed	Moderate	Intervarietal crossing allowed.	Caged with vs. caged without bees.	Arizona, USA	Pima S-5 *(G. barbadense)* and other unnamed	*Apis mellifera*

			wt./flower (20%)					varieties	
21	Kuliev	1958	Increased yield 25.9% for 2018-G and 23% for S-1472, boll weight 11-14%, weight of cotton 35-40%	Moderate	Allowed intravarietal crossing. Limited details on experiments, sourced from Avetisyan 1958	No detail provided	Azerbaijan, USSR	"Cotton" 2018-G, S-1472	Bees (ambiguous)
22	Saeed et al	2012	Bumblee bee pollination was better than open in terms of number of seeds per boll (MNH552: 9%, MNH633: 14%) and weight of seed per boll (MNH552: 15% MNH633: not sig).	Uncertain	Ambiguous if intervarietal cross were possible, and unclear how the effect of the greenhouse was accounted for in analysis. *Bombus* rather than *Apis* pollinators	Bee pollination in a greenhouse vs. hand vs. open pollination.	South Korea	MNH-552 and MNH-633 (*G. hirsutum*)	*Bombus terrestris*
23	Mahadevan & Chnady	1959	Increase of 23-53% in yield but no differences observed in quality, length, or ginning of fibre	Uncertain	Abstract only (obscure journal, cannot be sourced)	Open vs. caged to exclude bees, 2 plots.	India	Uncertain	Honeybees
24	Zheng et al	1981	Increase in ginned cotton yield (23%), summer boll number (39%), autumn boll number (19%), boll wt (17%), lint percentage (4%), seed number/boll (5%) and 1000-seed wt (3%) compared with controls.	Uncertain	Abstract only (obscure journal, cannot be sourced)	Caged with and without bees.	Zhejian, China	Uncertain	Honeybees
25	Berger et al	1988	Male sterile system: yield benefit not relevant	Low	Male-sterile hybrid seed production.	Caged with *Bombus* vs. caged with *Apis mellifera*	Texas, USA	Male-sterile *G. hirsutum*	*Bombus pennsylvanicus* vs. *Apis mellifera*
26	McGregor et al	1955	Male sterile system: yield benefit not relevant	Low	Male-sterile hybrid seed production.	Caged with bees vs. caged without	Arizona, USA	Pima (*G. barbadense*) S-1, A-44	*Apis mellifera*
27	Moffett & Stith	1972	Male sterile system: yield benefit not relevant	Low	Male-sterile hybrid seed production.	Caged with bees, different cage types	Mississippi, USA	Male sterile 3 spp. cross between *G. arboreum*, *G. thurberi* and *G. hirsutum* and a pollen source (isogenic B line)	*Apis mellifera*

28	Phillips & Simpson	1989	Male sterile system: yield benefit not relevant	Low	Male-sterile hybrid seed production.	Hives added, correlation between visits and boll set explored	Texas, USA	Mix of male sterile and male fertile lines	*Apis mellifera* and native insects
29	Vaissière et al	1984	Male sterile system: yield benefit not relevant	Low	Male-sterile hybrid seed production.	Hives placed beside fields	Texas, USA	*G. hirsutum* male sterile and male fertile lines	*Apis mellifera*
30	Vaissière	1991	Male sterile system: yield benefit not relevant	Low	Male-sterile hybrid seed production.	Comparison of different hive stocking rates	Texas, USA	*G. hirsutum* male sterile and male fertile lines	*Apis mellifera*
31	Waller et al	1979	Male sterile system: yield benefit not relevant	Low	Male-sterile hybrid seed production.	Bees inside cages	Arizona, USA	Mix of male sterile and male fertile lines	*Apis mellifera* and *Osmia fulviventris*

How large is the bee pollination benefit?

To be worthwhile promoting bee pollination as a useful part of practice the benefit from pollination must be enough to justify the management costs. None of the published studies proceed to a cost/benefit analysis, so the best that can be done is simply to examine whether the increases look substantial. It is important to note that the % benefit figures reported in the literature (table 1) are usually based on a comparison with zero insect pollination (i.e. plants in exclusion cages). This is appropriate in assessing the *total* value of insect pollination, however, the more useful figure for informing new practice is the comparison between improved practice and the status quo, which is open pollination with no added honey bee hives. In other words, one wants to know the *marginal* change associated with the new practice. This value will be smaller than the total, and will of course vary depending on the amount of free, unmanaged insect pollination experienced in the open treatment. Where free pollination is abundant the marginal benefit of additional pollinators might be zero. El Serrag et al (1993) report that while there was a significant benefit comparing caged with and without bees, open pollination was similar to "caged with bees", suggesting the background availability of pollinators in that landscape was high. In contrast, Wafa and Ibrahim (1960) report that "caged with bees" was superior to "open" in many measures, including a 25% increase in lint at plot scale, albeit in a study of *G. barbadense* (table 1). It might be expected that the amount of free pollination in a large, simultaneously flowering monocultural crop of cotton will usually be relatively low, because it is difficult for the landscape to support a large enough bee population to forage over this massive resource, but nevertheless relatively little is known about the actual amount of wild pollination occurring in these circumstances.

Keeping in mind the issue of total versus marginal values, the benefits reported across the whole range of studies are generally high, often on the order of 20% improvements in the yield of cotton lint (table 1). The data suggest that there is a contribution both in terms of the proportion of flowers that set bolls, and the number of seeds per boll which in turn increases amount of lint per boll. These reported benefits are large enough to justify further exploration of the potential to integrate managed pollination into practice.

Is beetle pollination significant?

Many beetles feed on pollen and are therefore found in flowers in sometimes high numbers. Chen et al (2011) refer to *Haptoncus luteolus* (Nitidulidae) as a cotton pollinator. Similarly, *Carpophilus aterrimus* (also in the Nitidulidae) were suggested as potential agents for cross pollination in Australian cotton fields by Llewellyn and Fitt (1996), but subsequent assessments of this idea have cast doubt upon the idea (OGTR 2008). There are plants with specialised beetle pollination systems, including crops that are pollinated by nitidulid beetles (e.g. Blanche and Cunningham 2005), but these flowers have traits that are distinctive to beetle pollinated flowers, in particular to maximise the chance of between-flower movements, which are much less frequent for beetles than for bees. From this point of view it seems unlikely that beetle pollination would play a substantial role for cotton, but the true test is to conduct an experiment. Chen et al (2011) support their claim that *Haptoncus* is a pollinator by reference to beetle morphology described in a Chinese masters thesis, which seems to imply no evidence from actual pollination experiments. Keshlaf (2008) did experiment with cotton plants caged with beetles, and found that the rate of boll set was comparable to plants that had all visitors excluded, indicating that beetles were not effective pollinators. I could find no papers that provide experimental evidence that beetles are responsible for a significant amount of cotton pollination anywhere in the world. It is also worth noting that both Rhodes (2002) and Keshlaf's (2008) experiments in NSW indicate that it was possible to elevate crop yield significantly by addition of honeybees even without controlling for the presence of beetles. This result suggests that the effectiveness of *Carpophilus* beetles as cotton pollinators in these fields was very low compared to what was achieved by bees.

How attractive are cotton crops for foraging honeybees?

The attractiveness of any crop to bees is determined by the traits of the crop in question, the alternative choices within foraging distance, and the condition of the hives (i.e. how strong is the colony demand for pollen or nectar?). Given this complex set of drivers one cannot expect a simple or universal answer to the question "how attractive is my crop?" But an assessment of literature can help set realistic expectations regarding the range of possibilities.

A number of studies indicate a view that cotton is relatively unattractive to bees. In some cases this is because observations were made and no bees were seen in the crop (e.g. Llewellyn and Fitt 1996). In some cases inferences are made on the basis of plant traits. For example, Keshlaf (2008) notes that many nectar gathering bee visits are focused on extra-floral nectar (rather than on flowers) and further writes, regarding the large spines on cotton pollen, that "It is likely that these characteristics make cotton pollen unattractive to honeybees." This kind of speculation can be tempting, and in the Keshlaf (2008) study was provided as a partial explanation for what were interpreted as low levels of pollen collecting (cf nectar collecting). However, field observations in other studies show that pollen collecting can sometimes occur at high levels (Danka 2005). This apparent contradiction serves as a warning regarding the risk of interpreting attractiveness on the basis of traits rather than by direct studies of field pollination behaviour. Further, it is no surprise that observational studies with relatively small sampling effort will sometimes see few or no bees, because abundance can be strongly influenced by the weather, local land management (especially pesticide application) and the density of managed hives in the landscape.

Moffett et al (1975) uses 4 years of observations to show a strong seasonal pattern in flower visiting by honey bees that ranges from highs over 20% (i.e. bees per 100 flowers) in early July and late October, but a low around 2% in August. Moffett et al (1975) nevertheless reports that underlying this average pattern is high variability among sites and years. Waller et al (1981) confirm the pattern of great changeability over time. In much smaller studies, Keshlaf (2008) records a range from 1.3 to 6.8% with a low point in the middle of the flowering period (with hives proved at 16 per ha in NSW) and McGregor (1959) reports honeybee visits ranging from 0 to 17% (with hives provided at 12 and 7 per ha in Arizona). As expected there is considerable variation among these studies in the actual numbers reported (and there are more studies with data like these), but given the vagaries of this kind of observational data, and the fact the data were collected from different continents and different times, what is more notable is that the figures are of the same order of magnitude.

It would be possible to compile a long list of studies with various records of bee visitation (including references from Appendix A). The list would include a wide range of different metrics (e.g. Gulati et al 2005 report 7.5 bees per m^2 per min, rather than bees per 100 flowers), would range across various cultivars and study locations, and would confirm the observation that the amount of bee activity varies by time and place. However, the important message from these studies is that honeybees *do* forage on cotton flowers, and at least in some cases *do so in considerable numbers*. Or in other words, low or zero numbers of bees at some times or in some places cannot be taken to indicate a consistent lack of attractiveness to honeybees. These studies do not give a clear direction regarding the *relative* attractiveness of cotton (versus other crops) or of the inclination of bees to forage on cotton when they have been placed near a crop in the interest of better pollination.

Data on pollen sampled from hives indicate that cotton is often less attractive than other crops in any given landscape (e.g. Waller et al 1981, Danka 2005, Keshlaf 2008) but bees are exploiting the crop nonetheless. El Sarrag et al (1993) observed honeybees on three different crops in the same study area (in Egypt) and observed that honeybees were more abundant on cotton than lucerne, but similarly abundant on sunflower. Berger et al (1988) observe that *Bombus* (bumble bees) were more abundant on cotton flowers than *Apis mellifera*, emphasising that different bee species will have different preferences.

The strongest indication of the inclination for honeybees to forage on cotton flowers come from the studies that examine whether bees placed on the crop provide a yield benefit. Whereas studies that force bees on to the crop by restricting them in cages do not inform us about relative attractiveness, many studies did link a yield benefit to free flying honeybees. Some report a correlation between visitation and boll set (e.g. Rhodes, 2002, Gulati et al 2005) others establish that open pollination flowers were bee visited, and yielded better than caged flowers (e.g. Moffett et al 1978, Tanda 1983 & 1984, El Sarrag et al 1993, Keshlaf 2008, Sanda et al 2013). Clearly if honeybees were generally reluctant to forage on cotton flowers then it would not be likely that all these studies would attribute a pollination benefit to the action of free flying honeybees.

In summary, while cotton may not be the most attractive crop for honeybees, it is attractive enough that they are often observed on flowers, often outnumber other flower visitors, and in many published studies their visits are enough to significantly raise fruit set relative to flowers that have not been visited. All plants are different in their relative attractiveness to honeybees, depending on the quantity and quality if the nectar and pollen they provide, and the amount of work bees have to do to get the reward. Beekeepers are quite aware of these differences, and have various management techniques that increase the amount of foraging that their bees will do on less attractive crops. Similarly, their willingness to exploit a particular crop at any given time depends on the choices available to them in the landscape, which vary year by year. It should be expected that bee keepers will continue to have an interest in using cotton crops as a resource in some years and in some locations. If managed pollination of cotton crops is to be encouraged, then beekeepers will expect a payment per hive that is determined in part by the quality of the resource for the bees (i.e. do my hives come off healthy?), the risks to their bees (i.e. is there a chance of accidental bee kills from insecticide?) and opportunity costs (how much money would I be making if I put my hives on one of the other species that flowers at the same time?).

Knowledge gaps

This analysis of the literature clearly establishes that honeybees will often exploit the nectar and pollen resources in cotton fields and in doing so they pollinate flowers and improve yield. Further studies are unlikely to change these general conclusions, but would instead simply add further examples, and add more observations to what is (like most ecological processes) a variable phenomenon. The critical next steps are not to ask “do honeybees pollinate?” but rather to move to the management question “can we increase crop productivity by incorporating insect pollination into cotton farming practice?” We do not yet know the answer to this question in part because not enough is known about the status quo (i.e. does free pollination maximise my yield?) but also because experiments to date have not usually focused on the costs and benefits of adding managed hives in a normal production environment. It is also critical to conduct experiments using the currently favoured varieties, because constant varietal improvements will change the degree to which there are any further benefits available from insect pollination.

Here I identify four research areas that would significantly advance our understanding of the potential for the cotton industry to benefit from honeybees.

(1) To establish the background level of free pollination one could examine the frequency of potential pollinators in the current production environment. A useful design that has been widely adopted in other studies is to count flower visitors in locations that might have high visitation (e.g. edges near remnant vegetation that may have wild pollinators) and compare with parts of the field relatively far from likely sources of wild pollinators (e.g. in the interior of large fields, e.g. Ricketts et al 2008). This can be matched with an assessment of boll set (flowers turned into bolls) along the same gradient. This kind of study can help establish if there is a pollinator deficit in parts of the field and can be taken a step further, using hand pollinations to see if the gap between *observed* and *potential* pollination outcomes differ according to location in the field (Cunningham 2014). These experiments give insight into free pollination and would help to understand the degree to which

honeybees are foraging in cotton crops in Australia, which is relevant to questions regarding pesticide exposure. But these experiments would not directly establish if provision of managed pollinators is likely to raise productivity for cotton growers.

(2) To understand the potential for cotton productivity benefits from using managed hives one can establish trials with and without managed honeybees, and compare the outcomes. This kind of experiment is more logistically challenging than the typical small plot experiments that are usually published, because it requires access to many replicated fields to establish representative “with” and “without” managed bee treatments, rather than using caged plots or bagged flowers to represent “without”. The “without managed bees” treatment can also be achieved by surveying a long gradient from a hive placement, stretching from the “near” hives end, where bees are abundant, to the “far” end, were bee density is lower. Other studies show that declines into flowering crops are expected on the order of a few hundred meters (Cunningham and Le Feuvre 2013, Ricketts et al 2008), but will depend on circumstances including the number and arrangement of hives (Cunningham 2014).

(3) If a full scale field trial using managed bees in conducted (i.e. project 2, above), then the results can be used to conduct a cost/benefit analysis. A good model for this approach is provided by Cunningham and Le Feuvre (2013). The benefit side requires a good understanding of the size of the increase in value created by cotton productivity increases, and how they relate to the number of hives required. It is also critical to understand the area over which benefits are achieved, and how this relates to the number and arrangement of hives. The cost side is driven by the per hive charge that beekeepers ask for hive provision. This would be informed by interviews with beekeepers. The goal of such an analysis is not to get a precise number, so much as to understand whether the cost/benefit equation suggests that paying for honeybee hives is likely to be economically beneficial for cotton growers.

(4) Fully integrating managed pollination into a production system is not achieved by simply bringing in hives. The productivity benefits associated with pollination will effect resource allocation by plants, timing of boll set and so on, which could change plant side demand for resources. Experiments in other crops have indicated yield benefits from crops without requiring increased nutrient inputs, but the longer term system management consequences are unknown. Use of honeybees would also place constraints on the application of certain insecticides at certain times (especially around flowering). Integration of managed pollination into production requires a better understanding of the potential whole-of-system changes. This would be achieved by consulting with agronomists and beekeepers and trialling improvements in practice by field experimentation.

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CONTACT US

t 1300 363 400
+61 3 9545 2176
e enquiries@csiro.au
w www.csiro.au

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CSIRO Agriculture Flagship
Saul Cunningham
t +61 2 6246 4356
e saul.cunningham@csiro.au
w http://www.csiro.au/Organisation-Structure/Flagships/Agriculture-Flagship.aspx