



Incidence of non-target pest species and validation of IPM strategies in Bt cotton hybrids deployed with different events of *cry* genes

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ABSTRACT

Cotton cultivars incorporated with different events of Cry proteins were evaluated for the incidence of sucking insect pests and abundance of predators under unprotected conditions during the years, 2009 and 2010. Among the sucking pests, whitefly, leafhopper and thrip were the major pests and there was no difference in the abundance of sucking pests among different hybrids. An integrated pest management (IPM) module based on use of eco-friendly strategies was developed and evaluated for transgenic cotton cultivars carrying different events including non-Bt cotton during 2010 and 2011 and was compared with the recommended regional package of practices (RPP) involving use of selective insecticides for sucking insect pests and bollworms. Results indicated low population of leafhopper, thrips and whitefly in IPM as compared to RPP. In addition, the cotton hybrids managed by IPM practices supported higher abundance of natural enemies. The incidence of cotton leaf curl disease (CLCuD) was also less in IPM as compared to RPP module. No bollworm incidence was recorded in any of transgenic cotton hybrid in either of the modules; however IPM and RPP with non-Bt hybrid showed rosette flowers and green boll damage due to pink bollworm, which was less in IPM as compared to RPP. IPM module resulted in low cost of insecticidal sprays and increase in C: B ratio compared to RPP. Furthermore, as the hybrids carrying different events did not differ significantly for the incidence of sucking pests, the IPM module developed was found effective for all hybrids including non Bt cotton hybrid with some revision in the light of bollworm incidence.

Key words: *Bt* cotton, *cry* genes, Events, Non-target pests

Cotton known as “whitegold” plays an important role in Indian economy. India is having maximum area under cotton and was grown on an area of 115.53 lakh hectares during 2013-14 {(ICAR-AICRP (Cotton) Annual Report-2013-14)}. During 2011-12, the area under cotton was 121.91 lakh hectares where out of 74-99% of it was under transgenic cotton in various cotton growing states of India (Kranthi 2012). As a highly selective form of host plant resistance, Bt cotton effectively controls a number of key lepidopteran pests and has become a cornerstone in overall integrated pest management (IPM). Cotton hybrids expressing various endotoxins of *Bacillus thuringiensis* Berliner for host-plant resistance have given a new dimension and impetus to the IPM philosophy that aims to reduce the massive reliance on insecticides for pest management on

the conventional or non-Bt cotton. Bollgard cultivars produce Cry1Ac toxin to control bollworms (Tabashnik *et al.* 2008, Kranthi *et al.* 2009). Bollgard-II cultivars combine two *Bt* Cry toxins (Cry1Ac and Cry2Ab) and offers an improved pest management compared to Bollgard cultivars (Kranthi *et al.* 2006, Vitale *et al.* 2008). Besides control of lepidopterous pests, transgenic cotton also offers the potential to decrease the use of broad-spectrum chemical insecticides (Fitt 1994, Lu *et al.* 2010) and promote relative safety for non-target organisms (Cheng *et al.* 2011, Li *et al.* 2011). Although reductions in insecticide use in some regions have elevated the importance of several pest groups, most of these emerging problems can be effectively solved through an IPM approach (Noranjo 2011). With the biotic potential of sucking pests being high, they are a potential threat to Bt cotton in the absence of broad-spectrum insecticides which are no longer in use. Other insects, both harmful and beneficial, which were also once suppressed by these insecticides, are now seen increasing in their abundance. In India, 1128 Bt cotton hybrids have been approved for commercial cultivation (Kranthi 2012), and a good number of them incorporated with different events of *cry* genes are under development. The study was aimed to record the incidence of sucking insect pests in Bt cotton hybrids

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incorporated with various events of Cry protein and validation of IPM module in light of the reaction of different Bt cotton hybrids to sucking pests.

MATERIALS AND METHODS

The incidence of sucking insect pests was studied in Bt cotton hybrids with different events, viz. MON 531 (*cry1Ac*), MON 15985 (*cry1Ac* and *cry2Ab*), Event 1 (modified *cry1Ac*) and GFM event (fusion *cry1Ab-cry1Ac*) under unprotected condition during 2009 and 2010 at Central Institute of Cotton Research (CICR), Regional Station, Sirsa. The Bt cotton hybrids carrying different events of *cry* gene Bioseed 6488 Bt, Bioseed 6488 BG-II, Non-Bt Bioseed 6488, JKCH 1050, NCEH 6 were planted under randomized block design (RBD) with four replications and the plot size was 5.4×4.0 m². The recordings of observations were started 45 days after sowing of the crop. The observations on sucking pests (leafhopper, whitefly, thrip/3 leaves and mealybugs per plant) were recorded from 5 tagged plants (same plants were observed at each observations) at fortnight interval starting from 45 days after sowing (DAS) of the crop. Similarly, the observations on the generalist predators were also recorded per plant.

IPM module was evaluated for Bt cotton hybrids deployed with different *cry* gene events during 2010 and 2011 crop seasons at experimental area of CICR, Regional Station, Sirsa following all recommended agronomic practices and it was compared recommended regional package of practices (RPP). The sowing for both IPM and RPP plots was done on 03.06.2010 and 21.05.2011 during both years.

The Bt hybrids carrying different event (Jai Bt, Jai BG-II, Jai non Bt, JKCH-1050 Bt, NCEH-6 Bt) were sown with

spacing of 100 × 60 cm² in a plot size of 1000 m² under each hybrid and was divided into five equal blocks to serve as replication for recording observations and to meet statistical analysis requirements. So, each module was laid out on an area of 0.5 ha (5 hybrids × 1000 m²) and separated by a row of sorghum with 1.5 m buffer area distance (Table 1). The seed was already treated with imidacloprid under both the practice but in IPM plot, the seed was also dressed with *Trichoderma viridae* @ 4 g per kg seed. The refugia were also sown both in IPM as well as RPP plot. The Bt IPM module comprised of two rows of sorghum sown around the boundaries to act as physical barrier for mealybugs and to increase the activity of natural enemies, installation of pheromone traps and application of neem oil as first intervention followed by *Verticillium lecanii* and need based use of selective insecticides for sucking pests and bollworms especially pink bollworm. RPP module was based mainly on chemicals, like imidacloprid 200 SL, acetamiprid 20 SP, triazophos 40 EC, ethion 50 EC, for sucking pests where as lambda cyhalothrin 5 EC and acephate 75 SP were applied against bollworms in no Bt genotype

Observations on the population and incidence of insect pests were recorded on 5 randomly selected plants in each block (total 25 plants per hybrid) at 15 days interval avoiding border rows. The percent incidence of CLCuD was also recorded. The incidence of bollworms, *Helicoverpa armigera* (Hubner) and *Earias* spp. was recorded on whole plant basis. The damage to fruiting bodies/ squares/flowers/ bolls was recorded based on the total number and damaged in each plant. The fruiting bodies both shed and intact on plants were taken into account for calculating the per cent damage. The observations on flower resetting, number of pink bollworm, *Pectinophora gossypiella* larvae per 25

Table 1 Detail of the interventions applied under IPM and RPP practices

Particulars	Bt IPM plot	Recommended regional practices
Hybrids	Jai Bt, Jai BG-II, Jai non Bt, JKCH-1050 Bt, NCEH-6 Bt	Jai Bt, Jai BG-II, Jai non Bt, JKCH-1050 Bt, NCEH-6 Bt
Size	1 acre	1 acre
Refugia	4 Rows of non Bt cotton	4 Rows of non Bt cotton
Border crop	2 Rows of Jowar	
Intervention for sucking pests	45-60 DAS 1 st spray neem oil @ 2.5 litre/ha 60-75 DAS 2 nd spray <i>V. lecani</i> (conidial count confirmed) @1.0 kg/ha 75-90 DAS 3 rd spray Acephate75SP @ 2.0 ml/l of water 90-105 DAS 4 th spray Buprofezin @ 2.0 ml/litre of water 105-120 DAS 5 th spray of triazophos @ 4.0 ml/litre of water	45-60 DAS 1 st spray : imidacloprid 17.8EC @ 250 ml/ha 60-75 DAS 2 nd spray : acetamiprid 25EC 200 g/acre 75-90 DAS 3 rd spray: difenthiuron 625g/ha 90-105 DAS 4 th spray: spiromesifen 625/ha 105-120 DAS 5 th spray: ethion 50EC @ 2000 ml/ha
Mealybug*	Cultural practices like weed removal/uprooting of infested plants or spot application of safer insecticide based on incidence of mealybug	Cultural practices and application of insecticides (profenophos) based on incidence of mealybug.
Management practices for pink bollworm**	Pheromone traps (2 traps/ acre) of PBW. Spray thiodicarb for PBW management after crossing ETL (8 moths/night/ trap for 3 consecutive nights)	Sprays of insecticides lambda cyhalothrin/ spinosad.

*No Incidence was recorded both under IPM and RPP,** A single spray was applied only in non-Bt hybrid under both the modules.

green bolls and per cent green bolls damage were recorded both in Bt IPM and in RPP blocks at 90, 120, 135 and 150 DAS. At the time of each picking, the number of good and bad opened bolls and locule damage were recorded from 25 randomly selected plants. The data was averaged and presented as good opened bolls and bad opened bolls per plant. Cotton yield was recorded from 5 randomly selected plots of $6 \times 5\text{m}^2$ from each demarcated replication both in IPM and RPP plot separately and from the entire block also. Later, the data was presented as seed cotton yield per ha for the respective module.

The data for studying the incidence of sucking pests in Bt cotton hybrids was analyzed through ANOVA using F - tests. The comparison of module IPM and RPP was done through paired 't'-test.

RESULTS AND DISCUSSION

Incidence of sucking insect pests in transgenic Bt cotton hybrids

Among the sucking pests, whitefly, leafhopper and thrip were the major pests during years, 2009 and 2010. On the basis of 6 observations recorded at fortnight intervals, the population of leafhopper varied between 0.68 to 1.20 and 3.60 to 4.43 per 3 leaves during 2009 and 2010, respectively. The population of whitefly varied between 7.10-9.41 and 4.91- 6.13 per 3 leaves during 2009 and 2010, respectively. The thrip population out-numbered whitefly and leafhopper population and it varied between 10.74-12.75 and 8.61 to 12.31 per 3 leaves in all the hybrids tested (Table 2). In general, the population of leaf hopper and thrip was more during 2009 as compared to 2010, however no significant difference was observed in the incidence of sucking pests among the different hybrids with respect to events compared.

The hybrids harboring maximum and minimum population of individual sucking pests were different for both the years. Based on pooled data of two years, leafhopper (2.57/3 leaves) and whitefly (7.45/3 leaves) population was maximum, whereas thrips were minimum (9.92/3 leaves) in Bioseed 6488 BGII (Table 2). The population of leafhopper (2.22/ 3 leaves) and whitefly (6.20/ 3 leaves) was minimum in Bioseed 6488 Bt and NCEH 6, respectively. The data recorded on generalist predator indicated no significant

differences among different hybrids and is more or less dependent on prey density. The present study including previous by (Men *et al.* 2003) and (Bambawale *et al.* 2004) reported that transgenic Bt cotton had no impact on the sucking pest population and consequently required suitable management strategies. Both non-significant (Sharma and Pampapathy, 2006, Channakeshava and Patil 2009, Rao *et al.* 2010, Vanitha and Banu in 2011) and significant (Abro *et al.* 2004 and Naveen *et al.* 2007) differences in incidence of sucking pests between transgenic and non-transgenic cotton were reported earlier in many studies.

Mann *et al.* (2010) also recorded similar non-significant densities of sucking insects and predators on Bollgard and Bollgard-II cultivars of different *Bt* events as well as conventional cotton cultivars as recorded in our studies.

Validation of IPM strategies

As the incidence of sucking pests were non-significant (Table 2) among Bt, Bollgard-II and conventional hybrids, the IPM modules developed were revalidated on the Bt cotton hybrids deployed with different events. Sucking pests mostly active were leafhopper, whitefly and thrip. No mealy bug incidence was recorded under both the modules. The leafhopper, whiteflies and thrip did not cross the economic threshold level (ETL) during most part of the season; however these varied among the modules with higher numbers in RPP plots (Table 3).

In general, the population of sucking pests was higher during 2010 in all the genotypes as compared to 2011 irrespective of the module adopted. As per the pooled data of 2010 and 2011, leafhopper, thrips and whitefly population ranged between 1.93 to 2.24, 4.21 to 7.77 and 4.09 to 5.64 per 3 leaves under IPM practices, respectively. The corresponding numbers in RPP were 2.28 to 2.71 for leafhopper, 7.10 to 8.20 for thrips and 4.97 to 5.66 for whitefly per 3 leaves on various hybrids. The population of sucking pests recorded was low in IPM practices over RPP during both the years except in few observations where the population of sucking pests was low in RPP compared to IPM due to use of broad spectrum insecticides. The mean population of lady bird beetle, green lacewing and spiders per plant was 0.22, 0.24 and 0.50 under IPM, whereas it was 0.06, 0.08 and 0.31 in RPP fields, respectively during both

Table 2 Incidence of sucking pests on Bt Cotton hybrids deployed with different events of Cry protein under unprotected conditions (Pooled data 2009 and 2010)

Hybrids (Events)	Population/ 3 leaves			
	Leafhopper	Whitefly	Thrips	
Bioseed 6488 Bt	MON 531 (cry1Ac),	2.22 (1.57)	7.10 (2.71)	12.30 (3.54)
Bioseed 6488 BG-II	MON 15985 (cry1Ac and cry2Ab)	2.57 (1.68)	7.45 (2.77)	9.92 (3.19)
JKCH 1050	Event 1 (modified cry1Ac)	2.24 (1.58)	6.57 (2.61)	12.53 (3.57)
NCEH-6	GFM event (fusion cry1Ab-cry1Ac)	2.56 (1.68)	6.20 (2.54)	12.12 (3.52)
Non-Bt Bioseed 6488		2.46 (1.65)	7.16 (2.72)	10.65 (3.30)
CD		NS	NS	NS
SE (m)		0.08	0.13	0.21

*Mean of 6 fortnight observations, ** Figures in parentheses are $\sqrt{x+0.25}$ transformation

Table 3 Mean percent reduction and sucking pest population in IPM over RPP and population of natural enemies' in different *Bt* Cotton events recorded after scheduled sprays during 2010 and 2011 in IPM (Two year pooled data)

Cultivar	No. of sucking pest/3 leaves (*Percent reduction over RPP)			No. of predators/ plant			CLCuD (%)	
	Leafhopper	Thrip	Whitefly	Lady bird beetle	Green lacewing	Spider	2010	2011
<i>IPM</i>								
JKCH 1050	2.15 (12.24*)	7.77 (5.24)	4.09 (27.74)	0.14	0.2	0.42	37.13	5.95
NCEH-6 Bt	1.93 (17.87)	4.23 (44.56)	4.63 (6.84)	0.2	0.27	0.41	29.66	14.95
Jai BG II	2.11 (22.14)	4.23 (46.39)	5.64 (-3.30)	0.19	0.19	0.54	21.99	10.11
JAI <i>Bt</i>	2.24 (2.18)	4.21 (40.70)	4.28 (15.08)	0.27	0.27	0.53	22.3	11.58
JAI non-Bt	2.07 (9.21)	5.01 (37.22)	4.25 (15.00)	0.32	0.3	0.64	21.09	15.38
Mean	2.1	5.09	4.58	0.22	0.24	0.5	26.43	11.59
<i>RPP</i>								
JKCH 1050	2.45	8.2	5.66	0.02	0.07	0.26	38.53	4.96
NCEH-6 Bt	2.35	7.63	4.97	0.07	0.08	0.29	19.95	14.1
Jai BG II	2.71	7.89	5.46	0.02	0.08	0.33	34.57	9.01
JAI Bt	2.29	7.1	5.04	0.05	0.07	0.37	31.3	13.97
JAI non-Bt	2.28	7.98	5	0.13	0.12	0.3	36.45	16.18
Mean	2.41	7.76	5.23	0.06	0.08	0.31	32.15	11.64
P-value	0.0275	0.0099	0.0873	0.0009	0.0008	0.0066		
Paired t-value/df	3.3906/4	4.6238/4	2.2537/4	8.9242/4	9.1422/4	5.1748/4		
Tabulated t value/df	2.77/4							

-ve figure indicate the increases in population

the years. The population of the generalist predators was significantly high in IPM practices over RPP. Among the generalist predators, spider population was maximum as it was least effected due to the use of insecticides compared to other predators. The mean per cent reduction in leafhopper population in IPM block varied from 2.18 to 22.14, the corresponding figures for whitefly were 6.84 to 27.74 per cent with exceptional increase in Jai BG-II (3.30%) while the thrip varied from 5.24 to 46.39 per cent over RPP.

In the modules IPM (based on ecofriendly strategies) as well as RPP (insecticide based), most of the interventions designed and applied were for the management of whitefly, a vector for CLCuD. The data recorded at the end of season for incidence of CLCuD during both the years revealed the higher incidence of CLCuD during 2010 as compared to 2011. The differential reaction among the hybrids was recorded during both the years regarding the incidence of CLCuD. But during both the years, the average incidence recorded was comparatively less in IPM as compare to RPP. The incidence recorded was 26.43 and 11.59 per cent under IPM and 32.15 and 11.64 per cent under RPP during 2010 and 2011, respectively. Irrespective of the modules, *Bt* genotypes deployed with different *cry* gene events registered significantly less population of bollworm due to the resistance afforded by *Cry* protein. No incidence of *H. armigera* and *Earias* spp. was recorded both on *Bt* and non -*Bt* cotton

hybrids in spite of the experimental farm having area under conventional cotton. The mines and warts available on the epicarp of bolls of each *Bt* cotton hybrid witnessed the failure attempt of pink bollworm to enter the boll. The locule damage due to pink bollworm and its larval recovery were noticed only in non *Bt* cotton hybrid (Table 4). The rosette flowers due to pink boll worm were recorded only in Jai non-*Bt* hybrid (0.75 in IPM) and (1.29 in RPP). No rosette flowers were recorded from any other *Bt* cotton hybrids at any stage of crop growth both under IPM and RPP during 2010 and 2011.

Table 4 Compiled mean of Rosette flowers, locule damage and PBW larvae in Jai non *Bt* ** under IPM and RPP plots (2010 & 2011)

DAS	No. of rosette flowers/plant		Locule damage (%)		Larval recovery	
	IPM	RPP	IPM	RPP	IPM	RPP
90	0.7	0.2	0	0	0	0
120	1.9	1.9	0	0	0	0
135	0.1	1.75	13.38	16.78	10.5	8
150	0.3	1.3	16.68	15.05	9	6.5
Mean*	0.75	1.29	7.52	7.96	4.88	3.63

**In *Bt* cotton hybrids rosette flower, locule damage and pink bollworm surviving larvae were not recorded.

The green boll damage due to pink bollworm was observed in Jai non Bt hybrid in both IPM and RPP with mean larval recovery of 10.5 and 8.0 at 135 DAS and 9.0 and 6.5 larvae at 150 DAS in IPM and RPP respectively. No green boll damage was observed in any other Bt cotton hybrids. The percent locule damage was observed more in RPP over IPM in Jai non- Bt (9.80 and 10.30 % at 135 DAS and 17.60 and 15.50 % at 150 DAS in RPP and IPM, respectively) during 2010 and (23.75 and 16.46 % at 135 DAS and 12.5 and 17.86 % at 150 DAS in RPP and IPM, respectively) during 2011. There was no significant difference observed in IPM and RPP in green boll damage and locule damage (Table 5). The IPM module was found effective in management of pink bollworm in non-Bt cotton hybrid along with its effectiveness against sucking pests.

The pooled mean of 2010 and 2011 recorded 32.68 and 29.57 good open bolls and 2.60 and 2.84 bad open bolls in IPM and RPP, respectively. The bad opening was not due to bollworm however it might be due to the rotting of bolls due to rains or other reasons (Fig 1).

Spray schedule and economics of IPM module

The average combined yield of two years from all hybrids was 7.77 and 6.50 quintal per acre in IPM and RPP plots, respectively. The total cost of cultivation/acre including insecticides and its application was ₹ 11 687.50 in IPM plots as against ₹ 11 930.50 in RPP plots. The net profit/

Table 5 Mines and warts observed on epicarp of green bolls in different Bt hybrids due to pink bollworm infestation.

Treatment	Mine/Warts (%)			
	135 DAS		150 DAS	
	RPP	IPM	RPP	IPM
Jai Bt	12	11.8	11.84	18.31
Jai BG II	10.63	11.82	14.82	15.21
Jai non-Bt	20.37	20.75	18.59	29.17
JKCH1050	8.32	7.2	11.65	16.31
NCEH6 Bt	7	10.92	9.7	18.34

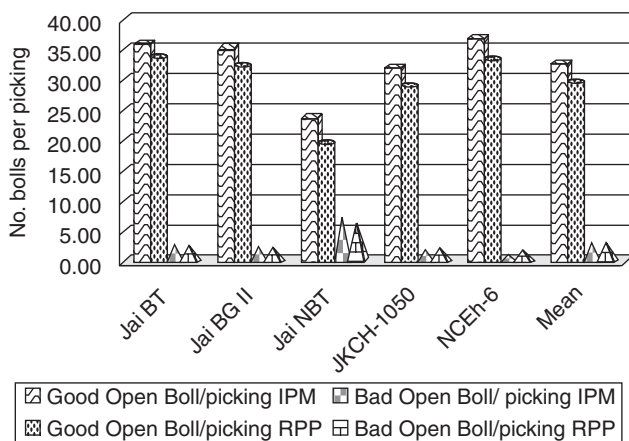


Fig 1 Average number of good and bad open bolls per picking in IPM and RPP plots. (Pooled date: 2010 and 2011)

acre was ₹ 23 277.50 in IPM and ₹ 18 647 in RPP, with a cost: benefit ratio of 1: 1.99 and 1:1.56 in IPM and RPP plots, respectively. The per cent increase in net profit in IPM was 24.83 over RPP (Table 6).

The IPM module mainly was based on eco-friendly strategies which reduced the population of sucking pests as compared to RPP. Low sucking pests population was recorded in validation of integrated pest management strategies for Bt cotton under rainfed ecosystem (Patil *et al.* 2011) as well as bio-intensive IPM (Shanmugam *et al.* 2006). Two early sprays of botanical (neem oil) as well as biopesticides (*V. lecanii*) provided protection from the sucking pests. Moreover, the population of generalist predators was also conserved under IPM, a serious concern after the introduction of Bt due to decrease in prey species. Similarly, the activity of generalist predators was also enhanced due to sorghum sown as physical barrier to mealybug under IPM as earlier reported by (Kumar *et al.* 2011) and (Durgaprasad *et al.* 2011). The present findings are in agreement with the reports of (Bheemanna and Patil 2003) who reported stem as well as shoot smearing of the cotton plants with imidacloprid was the best treatment in reducing the early season sucking pests without affecting the natural predatory population. In the present studies, the incidence of *H. armigera* was not recorded in any hybrid including non -Bt in either of the modules. (Wang *et al.* 2012) reported the halo effect in China where the pink bollworm population (91% eggs and 95% in larvae) was significantly reduced on non transgenic cotton along with the transgenic cotton. These results are comparable with the findings of (Bamabawale *et al.* 2004) and (Patil *et al.* 2004) who reported higher seed cotton yield in Bt cotton IPM plots compared to non-Bt IPM plots. Further, Venkateshalu (2005) and Udikeri (2006) reported that the modules comprising of Bt cotton were found to be superior with

Table 6 Details of spray, yield, C: B ratio and net profit in different hybrids sown under IPM and RPP (Pooled data of 2010 and 2011)

Details	IPM	RPP
Average yield (q/acre)	7.77	6.8
No. of spray	4	5.5
Cost of spray(₹)	1937.5	2180.5
Reduced cost over RPP (₹)	243	
Total income (₹)	34965	30577.5
Cost of cultivation (₹)	11687.5	11930.5
Net profit (₹)	23277.5	18647
*C : B ratio	1.99	1.56
Increase in net profit over RPP (%)	24.83	

Price of seed cotton hybrids: ₹ 4 500 /quintal; Net profit = Total income – (Cost of spray + ₹ 9 750 /acre for seed and other costs including picking); Cost of spray: Includes the cost of insecticides + machine and labour charges; Cost of cultivation: cost of spray + average cost of two years was ₹ 9 750 per acre for seed and other costs including picking); C: B ratio = Gross profit/Cost of cultivation

respect to seed cotton yield. The Bt cotton hybrids with different events of Cry protein do not exhibit any significant difference in the sucking pests incidence. The Integrated pest management module comprising of eco- friendly strategies was effective in reducing sucking pest's population but for non-Bt cotton hybrids, some revision in the light of bollworm incidence is needed.

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