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Biological performance of Bollgard II® Bt-resistant *vs* susceptible population of pink bollworm, *Pectinophora gossypiella* (Saunders) on non-toxic diet

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Abstract

Background The pink bollworm, *Pectinophora gossypiella* (Saunders), is a devastating global pest of cotton that has caused substantial economic damage to Bt Bollgard-II® cotton plants in recent years due to the evolution of Bt resistance. The associated fitness cost is assumed to be one of the factors delaying the development of resistance against Bt transgenic crops. Hence, the present study was undertaken to assess the biological performance of pink bollworms by comparing the life history and demographic parameters of a resistant (Field-R) and susceptible (Lab-S) population.

Results Prolonged larval duration (23.40 days in Field-R *vs* 18.80 days in Lab-S population), total life cycle (male = 50.00 *vs* 42.80 days; female = 53.60 *vs* 46.20 days), reduced fecundity (100.60 *vs* 154.20 eggs/female) and fertility (88.00 *vs* 138.00 fertile eggs/female) was observed. The demographic parameters indicated a significant reduction in the net reproductive rate (184.27 *vs* 276.72), innate capacity for increase in number (0.11 *vs* 0.15), finite rate of increase in number (1.12 *vs* 1.16 female progenies produced/female/day), weekly multiplication rate (2.16 *vs* 2.86), potential fecundity (545.06 *vs* 634.11 eggs), number of hypothetical F_2 females (33 955.65 *vs* 76 572.41), but longer mean length of generation (47.54 *vs* 37.74 days) and population doubling time (6.30 *vs* 4.62 days) in Field-R compared with Lab-S population. A stage-specific life table demonstrated the differences in survival rates between susceptible and resistant populations at various life stages, with the resistant population having higher generation mortality (0.22 *vs* 0.19).

Conclusions The study confirms the involvement of fitness costs associated with Bt resistance in *P. gossypiella*. Despite reduced reproductive fitness, the resistant population tried prolonging the larval stage as a compensatory mechanism to repair the damaged host tissues due to Bt intoxication and for accumulation of enough nutrient reserves for normal pupation and adult emergence. Presence of a high proportion of double Bt-resistant larvae in the field coupled with continued noncompliance with refug planting certainly favours the flaring up of this monophagous pest despite the observed fitness costs. The resistance cannot be effectively reversed unless suitable alternative management strategies are deployed.

Keywords *Bacillus thuringiensis*, Biological parameters, Compensation, Fitness cost, *Pectinophora gossypiella*

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Background

In recent decades, global agriculture has undergone substantial transformation with the introduction of genetically modified (GM) crops engineered to express insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt). These crops have been remarkably successful at reducing pest damage and enhancing crop yields, thereby alleviating pressure on conventional chemical insecticides. One of the most remarkable success stories is the cultivation of Bt cotton plants, which has substantially contributed to the sustainable management of several key lepidopteran pests, including the pink bollworm, *Pectinophora gossypiella* (Saunders) (Gelechiidae: Lepidoptera) (Tabashnik et al., 2023). However, the widespread deployment of Bt crops has faced challenges, most notably, the development of resistance in target pest populations (Naik et al., 2018; Tabashnik et al., 2019). This intricate evolutionary arms race between Bt crops and their target pests has raised concerns about the long-term sustainability of this technology.

The pink bollworm is a devastating global pest of cotton that inflicts substantial yield losses of 20%–30% (Fand et al., 2019). Deploying Bt cotton varieties expressing *cry* genes to target bollworm pests offered an effective and environmentally friendly management solution. Nonetheless, the emergence of resistance in *P. gossypiella* populations to Cry toxins in India has highlighted the need for a comprehensive understanding of the underlying mechanisms driving resistance evolution (Dhurua et al., 2011). The evolution of resistance to Bt toxins involves a complex interplay of genetic, ecological, and evolutionary factors that shape the fitness landscape of pest populations (Wang et al., 2019; Likhita et al., 2023). Fitness costs in insects result from the negative pleiotropic effects of genes that provide resistance (Groeters et al., 1994). They manifest when the fitness of resistant individuals is lower than that of susceptible individuals in the absence of toxins (Carrière et al., 2010). These fitness costs associated with resistance genes can significantly impact survival and development (Trisyono et al., 1997; Lisa et al., 2004, 2005), diapause (Lisa et al., 2004), and mating success (Groeters et al., 1994). Fitness costs have the potential to impede the evolution of resistance to Bt toxins and may contribute to delaying or preventing resistance (Carrière et al., 2001; Raymond et al., 2007). Assessing the fitness of individuals with resistance alleles in the absence of selection, such as on non-Bt cotton or refuge crops, aids in understanding resistance evolution (Carrière et al., 2001). Therefore, the deeper understanding of the life history traits and ecological dynamics of *P. gossypiella* is crucial for elucidating the factors influencing the evolution of Cry toxin resistance (El-Metwally et al., 2007). Life history traits, such as developmental rate, survival rate, fecundity, and longevity, play a pivotal role in shaping the population dynamics and evolutionary trajectories of insect pests (Brooks et al., 2017; Saeed et al., 2023). Understanding how these traits interact with Bt toxins and influence resistance evolution is essential for devising effective resistance management strategies.

Comparative life table analysis is a powerful tool that enables the comprehensive assessment of various life history traits in different populations of an insect species (Ning et al., 2017). This analysis provides valuable insights into the fitness costs and benefits associated with resistance to Bt toxins by shedding light on the trade-offs between resistance and other ecological factors. Hence, the present study critically identified and examined the fitness costs associated with double Bt resistance to understand its involvement in delaying Cry toxin resistance development in *P. gossypiella*.

Materials and methods

Collection and maintenance of *P. gossypiella*

A comprehensive survey was conducted in the major cotton growing region of Dharwad, Karnataka State, India (15.4889° N, 74.9813° E), during the crop season of 2022–2023. The boll samples collected from > 110 days old cotton plants (boll maturation stage) were subsequently brought to the Genomic Resources Laboratory of ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India (13.0206° N, 77.5887° E), for destructive sampling. The field collected larvae were reared indoors on a semisynthetic diet (Jothi et al., 2016) until their population stabilized for Bt resistance (Madhu et al., 2021). The field-collected pink bollworm larvae were transferred individually to plastic vials (6 mm diameter × 5 mm height) containing an approximately 1 cm^3 semisynthetic diet and labeled as Field-R population. Later, the male and female pupae were segregated based on the position of the genital and anal pores (Ramya et al., 2020). The emerged adult moths were released into oviposition jars at a 1:1 sex ratio. Fresh cotton shoots dipped in a glass vial containing water were provided as an oviposition substrate, and a piece of cotton leaf dipped in 10% honey solution was offered as adult food (Jambagi et al., 2023). Freshly hatched larvae were transferred individually into plastic tubes containing 0.5 mL of semisynthetic diet. The F_3 progenies obtained from this population were subjected to dose-mortality bioassays and life table analysis. A colony of pink bollworm maintained at ICAR-NBAIR, without exposure to any chemicals or Bt toxins since 2009 (National Accession Number: NBAII-MP-GEL-02a), was used as a susceptible control (Lab-S population). Further, it was also confirmed that the inbreeding depression due to sib-mating

in susceptible cultures was meager and negligible, as evidenced through reproductive performance (*i.e.,* fecundity and fertility of eggs). Both insect cultures were reared on a non-toxic semisynthetic diet for all experiments, except for bioassay studies. Both populations were maintained in a growth chamber at (25 ± 1) °C with 60%–65% relative humidity (RH) and a 14:10 h (light: dark) photoperiod.

Quantification of Bt toxins

The study aims to assess the relative resistance or susceptible level of *P. gossypiella* to Bt Bollgard II® cotton (Hybrid: MRC 7918 BG-II; Mahyco India Pvt. Ltd., Akola, India), which expresses combined Cry1Ac and Cry2Ab toxins, Bt seed powder containing both the Cry1Ac and Cry2Ab toxin proteins was used as a toxin source. Seeds were decorticated and ground to a fine powder in a motorized electric grinder. The powder was passed through a 250 µm sieve to ensure uniformity. Cry1Ac and Cry2Ab toxins from the seed powder were quantified via an enzyme-linked immunosorbent assay (ELISA) using a DesiGen™ Quan-T ELISA 96-well plate kit (Agrisure Diagnostics Llp, Jalna, India) following the standard protocol for replicated samples (triplicate). Each gram of Bt seed powder contained 3.25 µg of Cry1Ac and 182.32 µg of Cry2Ab toxins. The expression level of Cry2Ab in Bt seed powder was ~ 56 times higher than Cry1Ac. The same ratio was also maintained while preparing the different concentrations for dose-mortality bioassay. This was one of the prime reasons to use Bt seed powder as a toxin source over the purified toxins.

Dose-mortality bioassays

The field-collected pink bollworm larvae were reared in the laboratory for another three generations to stabilize the population and subsequently used for the experiment. As much natural mortality was observed in neonate larvae, early 2nd instar larvae from the F_3 generation of Field-R and Lab-S population were subjected to dose-mortality bioassays via the diet incorporation method, as described by Dhurua et al. (2011). The diet contained five appropriate concentrations (4.27, 1.43, 0.47, 0.15, and 0.05 g·kg^{-1} for the Field-R population; 0.31, 0.12, 0.04, 0.01, and 0.004 g·kg^{-1} for the Lab-S population) of combined Cry toxins (Cry1Ac + Cry2Ab), as well as a control (diet with non-Bt seed powder). These concentrations were determined based on the pre-bioassay bracketing, and the Cry toxin concentrations which gave 20%–80% larval mortality were considered for bioassay studies. Diet (1 mL) was poured into each well in a 128-well bioassay tray (C-D International Enterprises, Pitman, USA). Early 2nd instar individual larva was placed in each well using a small camel hair brush after the diet was air-dried under laminar air flow for 2 hours (Dhurua et al., 2011). The wells were covered with air-vented lids, and the bioassay trays were kept in a growth chamber for 7 days [temperature, (25 ± 1) °C; RH, 60%–65%; photoperiod, 14:10 h (light: dark)]. At each concentration, 30 larvae were tested, and the test was repeated thrice on alternate days. The observations were made 7 days after the treatment imposition as per the methodology described by Dhurua et al. (2011). However, the seven-day bioassay was adopted here in the experiment (Kranthi et al., 2004; Liu et al., 2017) to make sure that the treated larvae had not been infected by fungus, as Bt cotton seed powder was added to the semisynthetic diet. The observations were taken when the larvae reached the third instar and were recorded as live; otherwise, they were treated as dead.

Biological parameters

To assess the total number of eggs laid by female pink bollworms of both Field-R and Lab-S populations, fresh Bt cotton twigs placed in a glass vial containing water were kept in plastic jars (8.5 cm diameter × 14 cm height) and ten pairs of adults from Field-R and Lab-S populations were released into four jars, separately. Daily the twig was changed and the old twig was counted for the number of eggs laid. Further, the number of fertile eggs and percent hatching were calculated for both populations based on the number of eggs hatched, out of total oviposition. The remaining parameters such as egg, larval, pupal period, adult longevity, total lifecycle, preoviposition, and oviposition periods were recorded as per the standard protocol described by Ashok et al. (2020).

Life table studies

The different life cycle parameters of pink bollworms were recorded according to standard protocols to construct both stage-specific and age-specific life tables for Field-R and Lab-S populations. To construct an age-specific life table and record fertility traits, ten pairs of newly emerged adult pink bollworm moths were released into plastic jars (45 × 45 × 6 cm). Cotton twigs placed in glass vials containing water were kept inside the jar as an oviposition substrate, and cotton swabs dipped in 10% honey solution served as adult food. After sufficient oviposition, 250 eggs were collected cautiously with the help of a compound microscope and placed in ten separate plastic jars, each with 25 eggs, to maintain 10 replications.

Stage-specific lifetables for both Field-R and Lab-S populations were built by dividing the total life cycle of *P. gossypiella* into distinct developmental stages, such as egg, larva (I, II, III, and IV instar), pupa, and adult, by adopting the methodology explained by Deevey (1947) and Southwood (1978). The developmental time and survival or mortality for each life stage were subsequently

recorded. The observations were made daily at an approximate interval of 24 h, during which the number of hatched eggs, live and dead larvae were recorded to construct an age-specific life table. Similarly, mortality in each stage was noted to form a stage-specific life table. Adults that had emerged on the same day were caged together and allowed to mate and oviposit to construct an age-specific fecundity life table. The oviposition data were recorded until all the adult females died. According to Fisher's principle, the typical sex ratio is 1 (male):1 (female) for most insect species (Papach et al., 2019). Hence, the number of female births (m_x) was calculated by the number of eggs obtained per female divided by two.

Demographic parameters

The different parameters of the life fecundity table were determined by following the protocol explained by Howe (1953). They are as follows, x = pivotal age (days); s_x = the number of females surviving at the beginning of 'x'; dnx = the number of females dying between 'x' and 'x + 1'; s_0 = the initial number of individuals at the beginning of the study, *i.e.,* the number at age 0; mnx = the number of females produced; l_x = proportion of survivors (females) at the start of age interval 'x' (s_x/s_0); d_x = proportion dying during the age interval 'x' and 'x + 1' (dnx/s_0); $100q_x$ = rate of mortality during the age interval 'x' and 'x + 1'; L_x (life table age distribution) = {[$l_x + (l_x + 1)$] / 2}, alive individuals between 'x' and 'x + 1'; T_x (the number of individuals life days beyond 'x') = $\sum L_x$ (from 'x' to end); E_x (expectation of further life) = $(T_x/l_x) \times 2$; m_x = the number of female offspring born to a mother of age 'x'; $\sum m_x$ = gross reproductive rate; $l_x m_x$ = reproductive expectation; R_0 = net reproductive rate; T_c (approximate generation time) = $\sum x l_x m_x / R_0$; r_c (capacity for increase) = $\ln R_0 / T_c$; r_m (intrinsic rate of population increase) = $\sum e^{(-rmx).lxmx} = 1$; DT (population doubling time) = $\ln 2 / r_m$.

The values of 'x', 'l_x', and 'm_x' were derived from the information provided in the life tables. The sum of the products '$l_x m_x$' gives us the R_0, as described by Lotka (1925). Additionally, the R_0^2 was used to calculate the hypothetical number of F_2 females. The rate of female population growth per female per day, denoted as λ, was determined using the antilogarithm of the exponential rate of increase (*i.e.,* λ = antilog e^r_m). Using these data, the weekly population growth rate [$(\lambda)^w$] was calculated (here, w = generation time of pink bollworm in terms of the number of weeks). The stable distribution of different age groups of *P. gossypiella* was determined using the intrinsic rate of natural increase ($r_m = \ln (R_o/T_c)$), and the mortality rates for both the immature and mature stages were computed. The construction of the stable age distribution table followed the approach outlined by Andrewartha et al. (1954) and Atwal et al. (1974). The percentage distribution for each age group (denoted by 'x') was calculated by multiplying L_x by the exponential factor $e^{-r_m (x+1)}$. By aggregating the percentages across each stage, including the egg, larval, pupal, and adult stages, the projected percentage distribution was determined. The mortality-survival ratio (MSR) was calculated using the formula, $MSR = d_x/l_x$, and indispensable mortality (IM) was calculated as $IM = (1\text{-}l'_x/ l_x)$, where l'_x represents the number of individuals alive at the beginning of the age interval 'x' in the absence of the mortality factor.

Statistical analysis

Probit analysis was used to evaluate the dose-response mortality of both Lab-S and Field-R pink bollworm populations (Finney, 1971) using Polo-PC® 2.0 software (LeOra, 2002). Before the analysis, the larval mortality was corrected based on the survivorship in the control group using Abbott's formula (Abbott, 1925). The values estimated included the median lethal concentration (LC_{50}), the lethal concentration that kills 90% of the tested larvae (LC_{90}), and the model parameters (slope and intercepts). Goodness-of-fit statistics were calculated for each population. The resistance ratio (RR) was calculated by using the formula RR = (LC_{50} of Field-R population)/(LC_{50} of Lab-S population). The life table data were processed using a computer program created utilizing Microsoft Excel. The rest of the analysis and graphical representation was performed by using Tableau v2023.2 (https://www.tableau.com) and GraphPad Prism v10.0 (https://www.graphpad.com).

Results

Bioassay outcomes

The Lab-S and Field-R populations of pink bollworms responded differently to Bollgard-II® Bt cotton expressing Cry1Ac and Cry2Ab toxins. A high level of resistance (84.64-fold) was detected in the Bollgard-II® Bt cotton field-collected pink bollworm larvae ($LC_{50} = 0.931$; $\chi^2 = 5.334$; df = 3; $h = 1.781$) compared with the laboratory-reared population ($LC_{50} = 0.011$; $\chi^2 = 0.829$; df = 3; $h = 0.414$) (Table 1). Hence, the pink bollworm population collected from the field was designated the resistant population for comparison with the susceptible population via comparative life table analysis.

Biological parameters

The data on 12 life history parameters of *P. gossypiella* were compared between Lab-S and Field-R populations. The results showed that most of the evaluated parameters did not significantly differ from each other (Table 2). The

Table 1 Probit response of susceptible and resistant populations to Bollgard II® Bt cotton expressing Cry1Ac + Cry2Ab toxics

Population	LC_{50} (95% FL)	LC_{90} (95% FL)	Slope ± SE	χ^2	*h*	RR
Lab-S	0.011 (0.002–0.023)	0.105 (0.051–0.672)	1.317 ± 0.393	0.829	0.414	1.00
Field-R	0.931 (0.423–2.623)	4.843 (1.937–86.386)	1.790 ± 0.247	5.334	1.781	84.64

Lab-S laboratory-established Cry toxin-susceptible population, *Field-R* field-collected Cry toxin-resistant population; LC_{50} (95% FL) represents median lethal concentration in $g{\cdot}kg^{-1}$ (95% fiducial limits), LC_{90} (95% FL) represents concentration of toxin in $g{\cdot}kg^{-1}$ that kills 90% of the tested larvae (95% fiducial limits); *SE* standard error, *RR* resistance ratio, *h* heterogeneity

Table 2 Different biological parameters of the pink bollworm reared on a semisynthetic diet

Parameter No.	Particulars	Lab-S		Field-R	
		Mean ± SD	Range	Mean ± SD	Range
1	Egg/incubation period /days	4.80 ± 0.84[a]	4–6	5.80 ± 1.30[a]	5–8
2	Larval period /days				
	1st instar	2.00 ± 0.71[a]	1–3	2.20 ± 0.84[a]	1–3
	2nd instar	4.40 ± 0.55[a]	4–5	5.80 ± 1.30[a]	4–7
	3rd instar	5.40 ± 1.34[a]	4–7	6.60 ± 1.14[a]	5–8
	4th instar	7.00 ± 1.23[a]	6–9	8.80 ± 1.30[a]	7–10
	Total	18.80 ± 2.17[a]	17–22	23.40 ± 0.55[b]	23–24
3	Pupal period /days	7.40 ± 0.89[a]	6–8	9.60 ± 2.07[a]	7–12
4	Adult period /days				
	Male	11.80 ± 1.30[a]	10–13	11.20 ± 1.79[a]	9–13
	Female	15.20 ± 0.84[a]	14–16	14.80 ± 1.76[a]	13–17
5	Total life cycle /days				
	Male	42.80 ± 3.83[a]	40–49	50.00 ± 2.45[b]	47–53
	Female	46.20 ± 3.27[a]	42–51	53.60 ± 2.99[b]	50–57
6	Pre-oviposition period/days	2.80 ± 0.45[a]	2–3	3.20 ± 1.30[a]	2–5
7	Oviposition period /days	7.80 ± 1.30[a]	7–10	7.20 ± 1.21[a]	6–9
8	No. of eggs/female	154.20 ± 39.80[a]	96–206	100.60 ± 5.68[b]	95–110
9	No. of fertile eggs	138.00 ± 37.84[a]	88–191	88.00 ± 7.14[b]	81–99
10	Percentage of fertility	89.49 ± 4.98[a]	84–92	87.48 ± 3.42[a]	82–90
11	Percentage of pupation	88.00 ± 13.04[a]	80–100	86.00 ± 11.40[a]	70–100
12	Percentage of adult emergence	92.00 ± 8.37[a]	80–100	88.00 ± 8.37[a]	80–100

SD standard deviation; the same letter below the means represents do not differ significantly between the populations at $P < 0.05$ according to Tukey's test; *Lab-S* laboratory-established Cry toxin-susceptible population, *Field-R* field-collected Cry toxin-resistant population; the sample size was 250 eggs

incubation period (time between egg laying and hatching) in the Lab-S population was slightly shorter than that in the Field-R population. The laboratory-reared susceptible *P. gossypiella* completed its larval period faster than the Field-R population, and the difference is statistically significant. Regarding the total life cycle, the Lab-S population took a significantly shorter duration than the Field-R population. Moreover, significantly more oviposition (eggs/female) and fertile eggs were observed in the Lab-S population than in the Field-R population. The remaining parameters, such as the pupal period, adult period, pre-oviposition period, oviposition period, and percentage of fertile eggs, pupation, and adult emergence, were not significantly different between the Field-R and Lab-S populations.

Demographic parameters

The R_0 and potential fecundity were significantly greater in the Lab-S population than in the Field-R population, as shown in Table 3. However, the T_c was significantly greater in the Field-R population than in the Lab-S population. The hypothetical female population in the F_2 generation was 76 572.41 and 33 955.65 eggs per female in the Lab-S and Field-R populations of pink bollworms, respectively. Moreover, r_m and λ were slightly greater in the Lab-S population ($r_m = 0.15$;

Table 3 Life history traits of the pink bollworm

Population growth statistics	Formula	Calculated value	
		Lab-S	Field-R
Net reproductive rate (R_0)	$\sum l_x m_x$	276.72[a]	184.27[b]
Mean length of generation (T_c) /days	$\sum x l_x m_x / R_0$	37.74[b]	47.54[a]
Innate capacity for increase in number (r_m)	$\ln R_0 / T_c$	0.15[a]	0.11[b]
Finite rate of increase (λ)	Antilog $e^{r}{}_m$	1.16[a]	1.12[b]
Arbitary 'r_m' (r_c)	-	0.15[a]	0.11[b]
Weekly multiplication of population	$(\lambda)^w$	2.86[a]	2.16[b]
Doubling time (DT) /days	$\ln 2 / r_m$	4.62[b]	6.30 [a]
Potential fecundity (PF)	$\sum m_x$	634.11[a]	545.06[b]
No. of hypothetical F_2 females	$(R_0)^2$	76 572.41[a]	33 955.65[b]

Lab-S laboratory-established Cry toxin-susceptible population, *Field-R* field-collected Cry toxin-resistant population; the sample size was 10 pairs of pink bollworm; the different letters represent significant difference between the populations at $P < 0.05$ according to Tukey's test

λ = 1.16 females produced by one female per day) than in the Field-R population ($r_m = 0.11$; λ = 1.12 females produced by one female per day). The Field-R population could multiply 2.16 times per week under the given set of environmental conditions, whereas the Lab-S population could multiply 2.86 times per week. Nevertheless, the Field-R population required more time to double its original population size compared with the Lab-S population (Table 3).

Correlation studies

Correlation between the RR and life table parameters of pink bollworms was calculated (Table 4). Among the different biological parameters of *P. gossypiella*, egg period ($r = 0.992$), larval period ($r = 0.998$), pupal period ($r = 0.998$), total life cycle ($r = 0.998$ for male, 0.997 for female) and pre-oviposition period ($r = 0.950$) were significantly positively correlated with the RR. However, RR was negatively correlated with male adult longevity ($r = -0.976$), oviposition period ($r = -0.976$), percentage of adult emergence ($r = -0.999$), female adult longevity ($r = -0.949$) and percentage of fertility ($r = -0.909$). Among the demographic parameters, T_c ($r = 0.999$) and doubling time ($r = 0.997$) were significantly positively correlated with the RR, whereas significant negative corrections were observed with weekly multiplication of the population ($r = -0.982$), the number of hypothetical F_2 females ($r = -0.976$) and R_0 ($r = -0.938$).

Stage-specific life table

The survival individuals of *P. gossypiella* individuals from egg to adult emergence was 161 and 152, respectively, out of 250 eggs in the laboratory-reared susceptible population and field-collected resistant population (Table 5). According to the stage-specific mortality data ($100q_x$), the mortality rates of 11.60% and 16.00% were recorded in the egg stage for the Lab-S and Field-R pink bollworm populations, respectively. In addition, the lowest mortalities of 2.02% and 0.55% were recorded for the 3rd larval instars in the Lab-S and 2nd larval instars in the Field-R populations, respectively. The survival fraction (s_x) of

Table 4 Correlation between the resistance ratio and life table parameters of pink bollworm

Life table parameter	*r* values
Egg period	0.992**
Larval period	0.998**
Pupal period	0.998**
Male (Adult) period	–0.976**
Female (Adult) period	–0.949**
Total life cycle (Male)	0.998**
Total life cycle (Female)	0.997**
Pre-oviposition period	0.950**
Oviposition period	–0.976**
Fecundity	–0.806
Percentage of fertility	–0.909**
Percentage of pupation	–0.146
Percentage of adult emergence	–0.999**
Net reproductive rate (R_0)	–0.938**
Mean length of generation (T_c)	0.999**
Innate capacity for increase in number (r_m)	–0.290
Finite rate of increase in number (λ)	–0.289
Arbitary (r_c)	–0.281
Weekly multiplication of population	–0.982**
Doubling time (DT)	0.997**
Potential fecundity (PF)	–0.645
No. of hypothetical F_2 females	–0.976**

** Significant correlation at $P \leq 0.005$; *Lab-S* laboratory-established Cry toxin-susceptible population, *Field-R* field-collected Cry toxin-resistant population

Table 5 Stage-specific life table of the pink bollworm

Stages (x)	Survivors at beginning (l_x)		Mortality (d_x)		Survival proportion/%		Apparent mortality/100q_x		Survival fraction (s_x)		Mortality-survival ratio (MSR)		Indispensable mortality (IM)		log (l_x)		k value	
	Lab-S	Field-R	Lab-S	Field-R	Lab-S	Field-R	Lab-S	Field-R	Lab-S	Field-R	Lab-S	Field-R	Lab-S	Field-R	Lab-S	Field-R	Lab-S	Field-R
Egg	250	250	29	40	100.00	100.00	11.60	16.00	0.88	0.84	0.13	0.19	21.13	28.95	2.40	2.40	0.05	0.08
L_1	221	210	6	29	88.40	84.00	2.71	13.81	0.97	0.86	0.03	0.16	4.49	24.35	2.34	2.32	0.01	0.06
L_2	215	181	17	1	86.00	72.40	7.91	0.55	0.92	0.99	0.09	0.01	13.82	0.85	2.33	2.26	0.04	0.00
L_3	198	179	4	3	79.20	71.60	2.02	1.68	0.98	0.98	0.02	0.02	3.32	2.59	2.30	2.25	0.01	0.01
L_4	194	176	18	5	77.60	70.40	9.28	2.84	0.91	0.97	0.10	0.03	16.47	4.44	2.29	2.25	0.04	0.01
Pupa	176	171	15	19	70.40	68.40	8.52	11.11	0.91	0.89	0.09	0.13	15.00	19.00	2.25	2.23	0.04	0.05
Adult	161	152	0	0	64.40	60.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.21	2.18	0.00	0.00
K value																	**0.19**	**0.22**

Lab-S laboratory-established Cry toxin-susceptible population, *Field-R* field-collected Cry toxin-resistant population; L_1, L_2, L_3 and L_4 represent 1st, 2nd, 3rd and 4th larval instars, respectively; the sample size was 250 eggs

the Lab-S population of pink bollworms ranged from 0.91 to 0.98, whereas that of the Field-R population ranged from 0.84 to 0.99 at different life stages. The mortality-survival ratio (MSR = 0.13 and 0.19 in Lab-S and Field-R, respectively) and indispensable mortality (IM = 21.13 and 28.95 in Lab-S and Field-R, respectively) were found to be the highest at the egg stage in both populations. The generation mortality (k-value) was found to be greater for the Field-R population (k = 0.22) than for the Lab-S pink bollworm population (k = 0.19).

Age-specific life table

The age-specific fecundity of *P. gossypiella* was estimated to determine the survival of females (l_x) and the age schedule for birth at age x (m_x). The results revealed that the pre-oviposition period ranged from the 31st to the 33rd day in the Lab-S population (Fig. 1). In contrast, in the Field-R population, the pre-oviposition period ranged between the 40th and 43rd days of pivotal age (Fig. 1). The first batch of eggs was deposited on the 34th pivotal days ($m_x = 20.76$), and the process continued until the 45th day ($m_x = 7.81$) in the Lab-S population, with l_x of 0.59 and 0.05, respectively (Fig. 1). The susceptible female moths contributed the most to the mean progeny production ($m_x = 143.27$) on the 38th day of the pivotal day, which further followed a decreasing trend and attained the least contribution ($m_x = 7.81$) on the 45th day. On the other hand, females from the Field-R population started laying eggs on the 44th day ($m_x = 15.35$) and peaked contribution ($m_x = 119.20$) on the 48th day of the pivotal age (Fig. 1). Thereafter, a decreasing trend in egg laying was observed. The l_x during the oviposition period ranged from 0.55 on the 44th day to 0.09 on the 53rd day of the pivotal age in the Field-R population.

Age-specific distribution

In the Lab-S population, the adult stage contributed only 0.39% to the stable age structure, whereas immature stages such as the egg, larva, and pupal stages contributed 62.87%, 35.52%, and 1.22%, respectively (Fig. 2). Similarly, the egg, larval, and pupal stages contributed 59.94%, 37.92%, and 1.72%, respectively, to the stable age structure in the Field-R population. The adult stage contributed the least to the stable age structure in the Field-R population (0.43%) (Fig. 2). These results clearly exemplify that the immature stages of both pink bollworm populations contribute more to a stable age distribution.

Life expectancy

The life expectancy (E_x) of both populations of *P. gossypiella* declined with the advancement of development (Fig. 3), with newly laid eggs exhibiting E_x values of 12.53 and 14.46 days in the Lab-S and Field-R populations, respectively. At 45–50 days for the Lab-S population and 50–55 days for the Field-R population, which correspond to their respective pivotal ages, the E_x reached its lowest value of 2 days. Moreover, the gradual increase in the mortality rate (d_x) was confirmed by the decrease in the survival rate (l_x) of both susceptible and resistant populations of pink bollworm (Table 5; Fig. 4).

Survivorship curve

On a semilogarithmic scale, survivorship curve patterns of both Lab-S and Field-R populations of pink bollworm were plotted. The results showed that the survivorship curves for both populations closely resembled a type III curve (Fig. 5), indicating a consistent mortality rate across all developmental stages. Notably, there was a significant decline in survivorship during the early instar

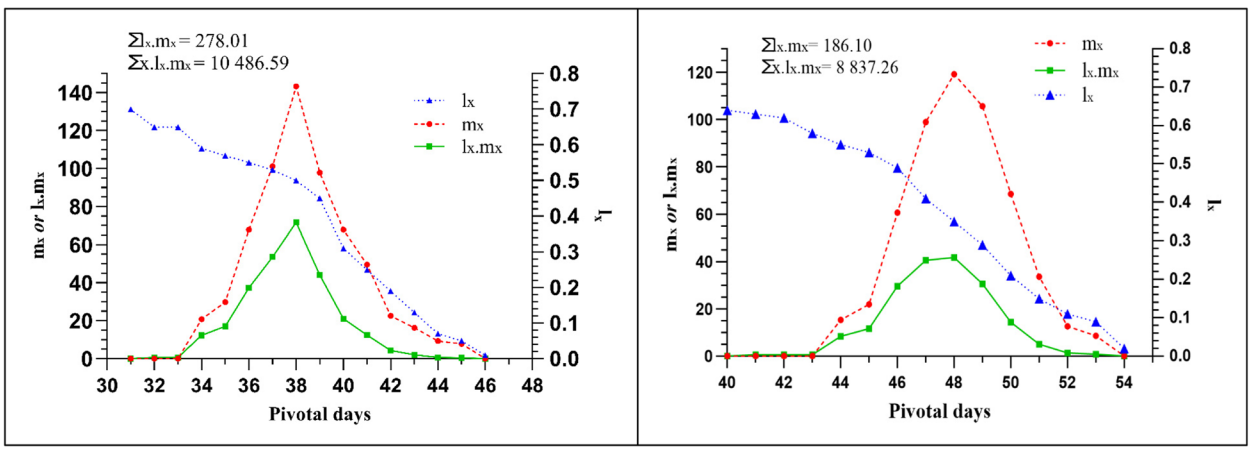

Fig. 1 The age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity (l_xm_x) of pink bollworm (Left figure represents Lab-S population; right figure represents Field-R population)

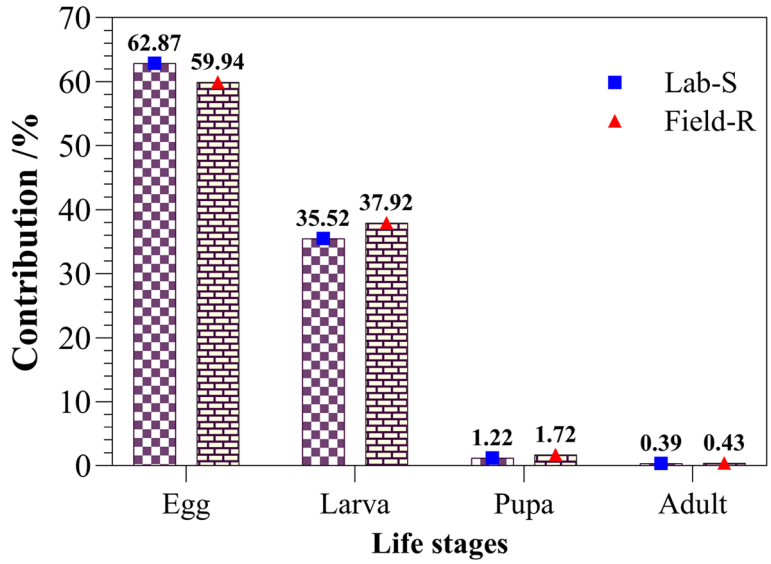

Fig. 2 Contribution of different life stages of pink bollworm to the stable age distribution

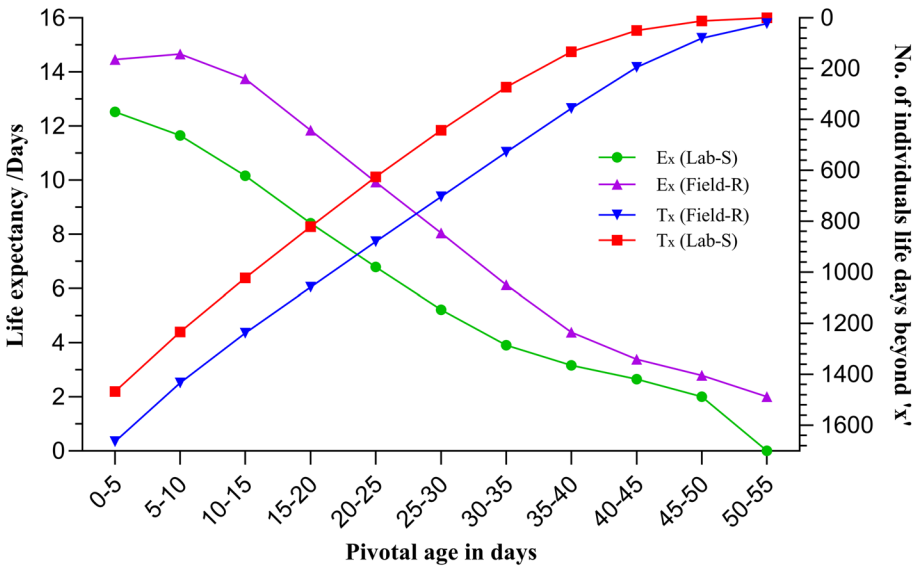

Fig. 3 Age-specific life expectancy of different population of pink bollworm (E_x: life expectancy in days; T_x: number of individuals life days beyond 'x')

larval stage, but overall, both curves displayed a steady decrease in survivorship as the pink bollworm reached the adult stage. These survivorship curves highlight the relatively high mortality during the early stages of the development compared to the later life stages of pink bollworm populations (Fig. 5).

Discussion

The field-evolved resistance of pink bollworm against Bollgard II® Bt cotton in India has resulted in 40%–95% boll infestation, with an estimated yield loss of 20%–30% (Fand et al., 2019). Considering the widespread infestation caused by the breakdown of Bt resistance, alternative strategies are being advocated to reduce pest damage and pesticide use (Nagrare et al., 2023).

Bt-resistant larvae tend to allocate a larger portion of their internal resources to combat the harmful effects of Cry toxins and insecticides. As a consequence, resistant populations collected from the field may frequently exhibit certain biological shortcomings when contrasted with susceptible populations that have been reared in laboratory settings. The biological response of

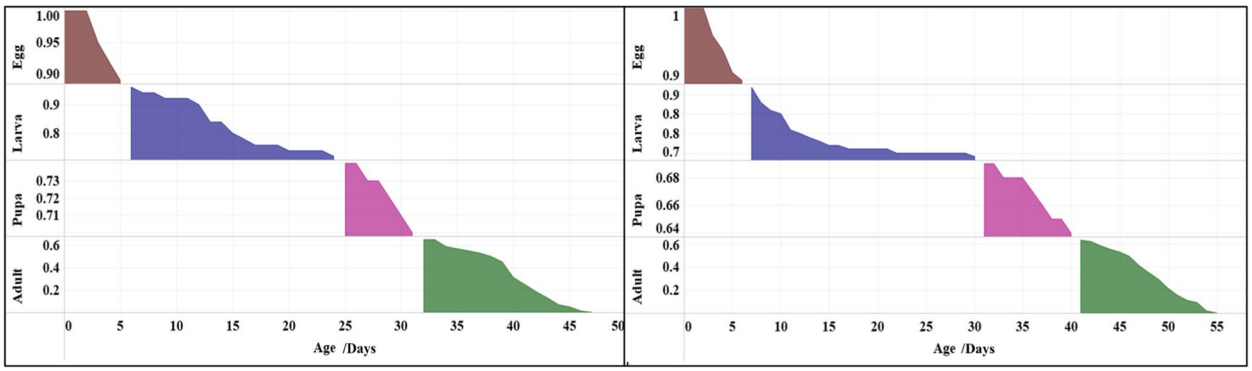

Fig. 4 Survival rate (l_x) at different life stages of pink bollworm (Left figure represents Lab-S population; Right figure represents Field-R population)

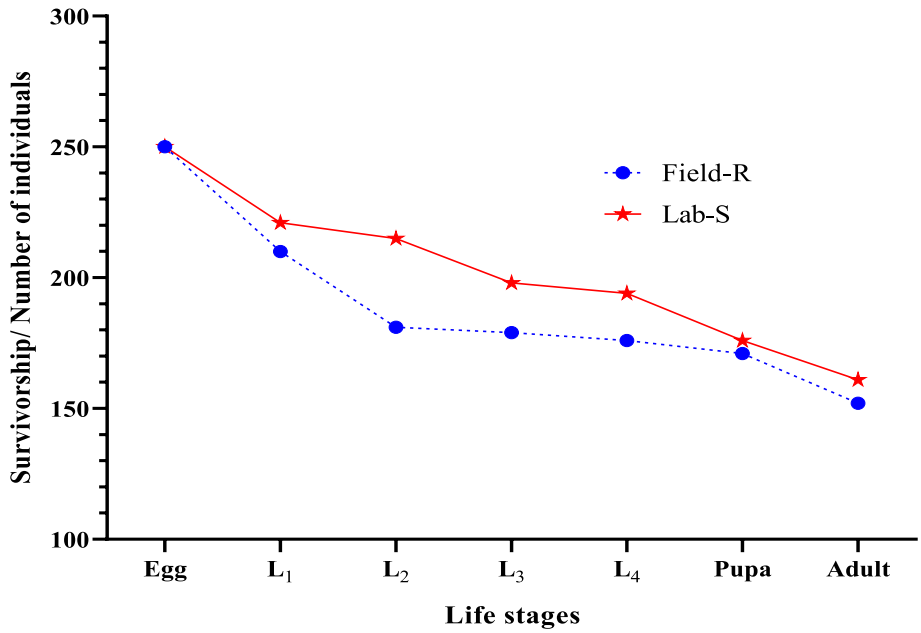

Fig. 5 Survivorship curves for Lab-S and Field-R populations of pink bollworm (L_1, L_2, L_3, and L_4 represent 1st, 2nd, 3rd and 4th larval instars, respectively)

a Bt Bollgard-II®-resistant field population and a laboratory reared susceptible population was examined in this study. The result revealed that out of the 12 biological parameters evaluated, only four (larval period, total life cycle, fecundity, and fertility) were significantly altered in the Bt Bollgard® II field-collected resistant population compared with the susceptible population. An extended larval duration is necessary for repairing damaged host tissues due to Bt intoxication and for accumulation of enough nutrient reserves for normal pupation and adult emergence. Studies suggest eight categories in which the host can respond to alleviate the negative effect of *B. thuringiensis* exposure, either to its pesticidal proteins or to the bacterium (Pinos et al., 2021). A previous study suggest that fitness costs are associated with Bt resistance in western corn rootworms and other insect pests (Gassmann, 2021). However, these small fitness costs did not play a major role in delaying the evolution of field-level resistance. Low fitness costs are the major cause of the evolution of practical resistance against Bt crops in many target pests (Carriere et al., 2023).

To alleviate the Bt intoxication effect, resistant larvae might have invested part of their energy in the detoxification process and host cell repair thereby diverting less energy for larval growth and development. It is also possible that prolonged larval duration is associated with juvenile hormone (JH) production, as previously reported in *Helicoverpa armigera* (Rao et al., 2008; Zhang et al., 2016). Several studies conducted on different

insects support this possible cause. The consumption of Bt protein by the noctuid moth led to elevated levels of JH, and it was deduced that this impact of the Bt protein was responsible for the prolonged development of the larvae (Pérez-Hedo et al., 2011). Furthermore, when *H. armigera* larvae were subjected to a one-day diet of Bt toxin, there was a minor increase in JH levels in their hemolymph (Muñoz et al., 2014). In the current study, the resistant population was exposed to Bt toxin in the field for numerous generations during the larval phase. Consequently, the increased JH levels in the resistant population may be a reaction to the stress caused by the Bt toxin, which in turn has repercussions on larval duration, total lifespan, and reproductive capacity.

The duration of egg to adult transition for both males and females of field-collected *P. gossypiella* was significantly longer than that for the susceptible population, which suggested that Cry toxin resistance is associated with fitness costs. Sayyed et al. (2001) reported that different populations of the same insect species can exhibit varying developmental times. They studied two populations of the diamondback moth, *Plutella xylostella*, one was resistant to insecticides and one was not. They found that the resistant population took longer to develop from egg to adult than the susceptible population. Similar results were also reported by Georghiou et al. (1977) and Roy et al. (2010), who found that resistant populations of arthropods often have longer development times than susceptible populations. This is considered a cost of resistance since the genes that make insects resistant to pesticides also negatively impact insect development. Additionally, our current findings can also be explained by the role of JH in influencing total longevity, as previously mentioned.

Several studies have explored the connection between resistance to Bt and its associated fitness costs, identifying fecundity and fertility as crucial factors influencing the selection for resistance (Liang et al., 2008; Cao et al., 2014). They are also critical aspects of insect biology and have broad implications for agriculture, ecology, pest management, and conservation. Studies related to fecundity and fertility can help us understand the strategies insects use to allocate resources to reproduction and how these strategies are influenced by environmental factors, food availability, and predation. Different insect species have their own fecundity rates (Vargas et al., 2015), as they have significant effects on the dynamics of the insect population (Jaleel et al., 2018). Nevertheless, this process also depends on several extrinsic factors, such as the quality of food and temperature (Lefkovitch et al., 1967). In our study, the Cry toxin-resistant population of pink bollworms exhibited significantly lower fecundity and fewer fertile eggs compared with the laboratory-reared susceptible population (Table 2). Groeters et al. (1994) and Sayyed et al. (2001) reported that Bt-resistant *Plutella xylostella* females produced fewer eggs than susceptible females. Campanhola (1988) reported that resistant *H. armigera* females produced 1 200 eggs each, while 2 500 eggs were produced per susceptible female. This finding suggested that the reduced fecundity of resistant females may be a consequence of the associated fitness cost of metabolic resistance to Cry toxins. Zhang et al. (2016) also reported a significant reduction in oviposition in Bt-resistant *H. armigera* populations compared with the susceptible population. One possibility for the reduction in the rate of egg laying in the resistant population may be due to less energy being partitioned to reproduction, as the major share of metabolic energy is likely allocated to developing biochemical and physiological defenses related to the detoxification of insecticides. Another possibility is the influence of JH-III on adult oviposition, as reported in previous studies, which suggested that the decline in fecundity might be attributed to increased JH levels, although the specific mechanism by which JH leads to a decrease in egg production when exposed to stress from Bt toxin remains unclear. However, JH affects the production and absorption of vitellogenin in the majority of insects, and alterations in hormone levels can impact egg production rates (Raikhel et al., 2005). Moreover, there are reports indicating that when male-derived JH is transferred to female insects, it can induce organizational changes that affect processes such as oogenesis, sexual readiness, and egg laying rates (Park et al., 1998; Pszczolkowski et al., 2006). Zhang et al. (2015) observed that the resistant strain of *H. armigera* exhibited reduced ovarian length, ovarian weight ratio, and follicle development stage. As a result, we inferred that the reduced fecundity of the resistant strain of *P. gossypiella* was also linked to the impact of the Bt toxin on ovarian development.

Life table parameters are important for understanding the population dynamics of insects. These methods can be used to predict population growth, identify key factors that affect insect survival, and develop strategies for pest management (Price, 1997). In the present study, the net reproductive rate and intrinsic rate of increase were higher in the susceptible population, whereas the mean length of generation time and population doubling time were greater in the field-collected resistant population of *P. gossypiella*. Furthermore, the finite rate of increase in number, weekly population multiplication rate, potential fecundity, and the number of hypothetical F_2 females were greater in the susceptible population than in the resistant population of pink bollworms. These findings clearly indicate that the fitness of the field-collected resistant population of *P. gossypiella* is compromised by the adverse effects

of lethal Cry toxins. Alyokhin et al. (1999) conducted an experiment in the Colorado potato beetle to compare the relative fitness of Cry toxin (Cry3A)-resistant and Cry3A-susceptible populations, and found that fitness parameters such as the net reproductive rate and intrinsic rate of increase were substantially lower in the resistant strain than in its counterpart susceptible population. In addition, we confirmed the involvement of fitness costs in Cry toxin-resistant population growth through correlation studies between different biological and demographic parameters and the resistance status of the pink bollworm (Table 4).

Furthermore, the age-specific distribution of both populations indicated that immature stages (egg and larva) was significantly associated with a stable age distribution (Fig. 2). The age-specific distribution of *Spodoptera litura* (Gedia et al., 2008) and *S. frugiperda* (Ashok et al., 2020) populations in previous studies also substantiated the maximum contribution of immature stages to a stable age distribution, which is consistent with the results of this study. The life expectancy of both resistant and susceptible populations decreased with increasing pivotal age (Fig. 5) and mortality rate, which was indicated by a decrease in the survival rate (Fig. 4). Life expectancy studies conducted on *Spodoptera litura* (Maghodia et al., 2008) and *S. frugiperda* (Ashok et al., 2020) also exemplify the decrease in E_x value as development progresses.

Conclusion

The results of this study confirm that the Bt-resistant field population of pink bollworms exhibits a generally slower growth rate compared with the susceptible population when reared on a non-toxic diet. The findings also underscore the importance of considering fitness costs associated with Bt resistance in developing sustainable pest management practices. Furthermore, the study highlights the need for integrated pest management strategies that mitigate the evolution of resistance while preserving the efficacy of Bt crops. Future research should focus on elucidating the molecular and physiological mechanisms underlying these fitness costs to enhance our understanding and management of resistance in pest populations.

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Authors' contributions
Jambagi SR: investigation, original draft preparation, methodology, software, data curation. Mohan M: conceptualization, formal analysis, project administrator, supervision, draft reviewing and editing. Muralimohan K: draft review and editing, formal analysis. Kambrekar DN: insect culture supply, formal analysis, visualization, draft reviewing and editing. Venkatesan T: formal analysis, draft reviewing and editing.

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Data availability
All the data obtained in the investigation have been incorporated into the manuscript and are available from the first author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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