

Efficacy of *Helicoverpa armigera* Larvae on Conventional and Genetically Modified (Bollgard®) Cotton plants from Marathwada Region (MS) India

S.S. Shinde¹, V.N. Kamtikar¹, Sumit Muley¹ and R.K. Nimbalkar²

¹Department of Zoology Vivekanand College, Aurangabad (MS) India

²Department of Zoology, Vinayakrao Patil College, Vajapur (MS) India

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*Corresponding Author

Tel : +91 9422345234
Fax : +91-2436224581

Email:
rkimbalkar@gmail.com

Abstract

Present study was conducted to evaluate cotton bollworm larval behaviour on Bollgard® cotton plants compared to conventional plants (Non-Bollgard®) and to determine their preferred feeding sites. Data from these studies conducted during pre-flowering and flowering stages are presented. The movement of *Helicoverpa armigera* was different on Bollgard® cotton plants compared to conventional cotton plants at all rating intervals. Cotton plant type ($F=25.47$; $df=1,10$; $P<0.01$) and time of evaluation ($F=54.15$; $df=3,30$; $P<0.01$). In pre-flowering cotton plants, bollworm larvae moved significantly more on individual flowering Bollgard® plants compared to conventional plants. Cotton plant type ($F=41.70$; $df=1,10$; $P<0.01$), time of evaluation ($F=6.79$; $df=3,30$; $P<0.01$), and the cotton plant type by time of evaluation interaction ($F=3.63$; $df=3,30$; $P=0.02$) was significant for percentages of larvae recovered from sticky traps. The study reports that current scouting protocols and action levels to initiate insecticide treatments for bollworms on non-Bollgard cotton plant are not appropriate for Bollgard® cotton plant. Scouts should look at white flowers and small bolls in addition to terminals and squares when scouting Bollgard® cotton plant because bollworm larvae migrate to those structures in a relatively short time.

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Key Words: *Helicoverpa armigera*, Efficacy, Bollgard® cotton plant, Marathwada region

Introduction

In India, Genetically Modified (GM) plants are rapidly becoming important components of integrated pest management (IPM) programs in many cropping systems. Cotton plant cultivars (Bollgard®, Monsanto Co., USA) that have been genetically modified to express the Cry1Ac protein from the soil bacterium, *Bacillus thuringiensis* Var. *kurstaki* Berliner (Perlak et al. 1990) are environmentally friendly tools for selective pest management and provide a significant economic return in many areas of India. In USA, Bollgard® cotton plant was introduced for commercial production in 1996, and in India it was introduced in 2002. Numerous lepidopteran pests include *Helicoverpa virescens* (F.); *Helicoverpa armigera* (Hubner); and *Pectinophora gossypiella* (Saunders), are susceptible to the Cry1Ac protein in Bollgard® cotton plants (MacIntosh et al. 1990, Luttrell et al. 1999). Bollgard® cotton plants have continued to provide satisfactory control of *Helicoverpa virescens* and *pectinophora gossypiella* populations and suppress other lepidopteran pests when densities are low to moderate. However, when high population densities of bollworms persist for several days, supplemental control with insecticides is often needed to prevent economic injury (Stewart et al. 2001).

Helicoverpa armigera, bollworm larvae are commonly observed in white flowers of Bollgard® plants (Smith 1998, Pietrantonio and Heinz 1999). In a laboratory bioassay, Stewart et al. (2001) observed 10 to 48% survival of bollworm larvae on Bollgard cotton plants flowers and bolls at 4 days. In

field tests, *Helicoverpa armigera* feeding caused 55% abscission of bolls infested at anthesis (Gore et al. 2000). Ovipositional preferences of *Helicoverpa armigera* bollworm between Bollgard® and conventional cotton plants have been evaluated as a possibility. Differences in sites of oviposition would not be expected between Bollgard® cotton plants and conventional (non- Bollgard) cotton plant because the Cry1Ac protein in Bollgard® cotton plant does not affect bollworm adults (MacIntosh et al. 1990). Furthermore, the morphology of Bollgard® cotton plants should be similar to the parental conventional breeding lines. Parker and Luttrell (1998) found no differences in *Helicoverpa virescens* (tobacco budworm) egg density or vertical distribution of eggs on plants of Bollgard® cottons compared with the conventional parental cottons. Similarly, no differences in sites of bollworm oviposition were detected on Bollgard® cotton plant compared to conventional cotton plant (Roof et al. 2001). In India all three *Bt* cotton genotypes significantly reduced the percentage of terminal regions containing live heliothine larvae compared to the non-*Bt* cotton plant genotype (Nimbalkar et al. 2008).

Dispersal of early instar bollworm larvae may be different on Bollgard® cotton plants compared to conventional cotton plants. According to Jyoti, 1996, in laboratory bioassays, bollworm larvae moved from cotton plant leaves treated with foliar *Bacillus thuringiensis* formulations and were found at other locations in the test arena. Also, bollworm larvae avoided feeding on meridic diets containing purified *Bacillus*

thuringiensis proteins or lyophilized Bollgard® plant tissues (Greenplate et al. 1998, Akin et al. 2001). Tobacco budworm, *Helicoverpa virescens* larval movement has been observed to be different on Bollgard® cotton plants compared to conventional cotton plants in field and greenhouse studies (Benedict et al. 1993, Parker and Luttrell 1999).

In both of these studies, *Helicoverpa* larvae moved from Bollgard® plant terminals more rapidly than from conventional plant terminals. However, the fate of larvae after leaving the terminals was not reported. Larvae at the developmental stage, is controlled by the Cry1Ac protein in Bollgard® cotton plant, and differences in larval behaviour could result in feeding preferences on specific plant parts. Terminal foliage expresses higher levels of Cry1Ac than other plant parts (Greenplate 1999, Greenplate et al. 2000). Levels of Cry1Ac expression in terminal foliage and fruiting forms on node nine averaged 68.1 and 26.5 µg/g dry weights, respectively (Greenplate 1999). In a similar study, Cry1Ac expression was higher in white flowers compared with squares and bolls (Adamczyk et al. 2001). Although protein expression was not measured in foliage, Cry1Ac expression was higher in bracts compared to flowers, squares, and bolls (Adamczyk et al. 2001). Variation in protein expression among different plant parts combined with bollworm detection and avoidance of the protein could result in bollworm populations becoming established on those structures with low protein expression. Field studies were conducted to evaluate cotton bollworm larval behaviour on Bollgard® cotton plants compared to conventional plants and to determine their preferred feeding sites. Data from these studies conducted during pre-flowering and flowering stages are presented.

Materials and Methods

Blocks (16 rows x 100 ft.) of a Bollgard® cotton plant cultivar (Rasi 134, Rasi Seeds (P) Ltd) and a conventional parental cotton cultivar (NHH 44, Mahindra Hybrid Seeds Co. M.S., India) were planted at the Village Vihamandwa, Thasil Paithan of Aurangabad district from 10th June during 2009 and 2010. Fertilization rates and general agronomic practices for cotton production followed current CCI (Cotton Corporation of India) recommendations. Bollworms were collected from clover, *Trifolium* spp., during April and sweet corn, *Zea mays* L., during month of June. Colonies were maintained in the laboratory for at least one generation to eliminate parasitoids, minimize pathogens, and obtain sufficient numbers of larvae at the proper stage for infestations on cotton plants. Larvae were fed a wheat germ/soy protein diet until pupation. Adults were held in 3.79-L cardboard containers and fed a 10% sugar-water solution. A single layer of cheesecloth was placed over the containers to provide an adequate surface for moth oviposition. Egg sheets were harvested daily and placed into plastic bags until larval eclosion. Upon eclosion, larvae were fed meridic diet in 236-ml cups (50larvae/cup) for 48hrs. After 48±3hrs, bollworm larvae were placed in the terminals of cotton plants during vegetative or reproductive developmental stages.

Infestation of Pre-flowering Cotton Plants

Individual Bollgard® and conventional cotton plants were isolated by removing all adjacent plants before infestation so that no interplant movement of larvae could occur. A single *Helicoverpa armigera* larva (first instar, 48±3hr old) was placed

in the terminal of a cotton plant with a small paintbrush. A 40.6-cm x 40.6-cm sticky trap was placed on the soil surface at the base of each infested plant. Sticky traps were used to recover larvae that apparently left plants by spinning-down on a silken thread. This experiment consisted of twelve replications over two years (2009 and 2010) in a completely randomized design. Replications were represented by day of infestation and 25 plants of 51 Bollgard® and conventional cotton plant were infested each day. Numbers of larvae recovered from sticky traps and remaining in plant terminals were recorded at 1hr, 3hr, 6hr, and 24hr after infestation. Data were converted to percentages based on the number of plants infested on a given day and analysed using repeated measures analysis of variance (SAS, PROC MIXED, Littell et al. 1996).

Infestation of Flowering Cotton Plants

First instar bollworm *Helicoverpa armigera* larvae (48±3hr old) were infested on individual Bollgard® and conventional cotton plants (1larva/plant) during flowering growth stages in 2009 and 2010. Individual plants were isolated by removing all adjacent plants before infestation so that no interplant movement could occur. Procedures and experimental design for larval infestations were similar to those described for pre-flowering cotton plants except sticky traps were not used. *Helicoverpa armigera* infested plants were examined at 3hr, 6hr, and 24hr after infestation. The number of main stem nodes that a larva moved from the plant terminal and plant structure (terminals, squares, flowers, bolls) infested with a larva was recorded. Data were analysed by using repeated measures analysis of variance (SAS, PROC MIXED, Littell et al. 1996).

In addition to single plant infestations, micro-plots (1rowx1m) were established within blocks of Bollgard® and conventional cotton plant cultivars during 2009 and 2010. Plants in micro-plots were infested with 20 first instar bollworm larvae. *Helicoverpa armigera* Larvae were placed in the terminals of plants using a small paintbrush and were evenly distributed across all plants within the micro-plots. A total of 20 and 25 micro-plots were infested during 2009 and 2010, respectively, for conventional and Bollgard® cotton plant. The experimental design was a randomized complete block and dates of infestation represented blocks. Whole plants within each micro-plot were inspected at 24hr and 48hr after infestation. Plant, square, flower, and boll densities were recorded from each micro-plot. Numbers of plant terminals, squares, flowers, and bolls infested with *Helicoverpa armigera* larvae were recorded. Data were converted to percentages of infested structures and analysed by using repeated measures analysis of variance (SAS, PROC MIXED, Littell et al. 1996).

Results

The movement of *Helicoverpa armigera* was different on Bollgard® cotton plants compared to conventional cotton plants at all rating intervals. Cotton plant type ($F=25.47$; $df=1,10$; $P<0.01$) and time of evaluation ($F=54.15$; $df=3,30$; $P<0.01$) effects were significant for the percentage of larvae remaining in cotton plant terminals (Figure1). More larvae remained in the terminals of conventional cotton plants compared to Bollgard® cotton plants at all rating intervals. At 1hr, 3hr, and 6hr, 47.8, 39.4, and 20.9% of larvae, respectively, remained in the terminals of conventional cotton plants. In

contrast, only 28.7, 11.4, and 6.3% of the larvae remained in Bollgard® cotton plant terminals at 1hr, 3hr, and 6hr, respectively. Within 24hr, nearly all (98.7%) larvae had left the terminals of Bollgard® cotton plants while 87.0% of the larvae left the terminals of conventional cotton plants. Cotton plant type ($F=41.70$; $df=1,10$; $P<0.01$), time of evaluation ($F=6.79$; $df=3,30$; $P<0.01$), and the cotton plant type by time of evaluation interaction ($F=3.63$; $df=3,30$; $P=0.02$) was significant for percentages of larvae recovered from sticky traps (Figure 1). Higher percentages of bollworm larvae were observed on sticky traps beneath Bollgard® cotton plants compared to traps beneath conventional cotton plants at all

rating intervals. At 1hr after infestation, 17.8% of the total numbers of larvae placed on Bollgard® cotton plant were recovered on sticky traps beneath plants compared to 6.1% beneath conventional cotton plants. At 3hr after infestation, 36.6% of the total number of bollworm larvae placed on Bollgard® cotton plants was found on sticky traps compared to 7.6% on conventional plants. At 6hr after infestation, 41.4% and 10.1% of the total number of larvae were recovered from sticky traps beneath Bollgard® and conventional cotton plants, respectively. At 24hrs after infestation, 46.3% of the larvae were recovered from sticky traps beneath Bollgard® cotton plants compared to 10.5% beneath conventional cotton plants.

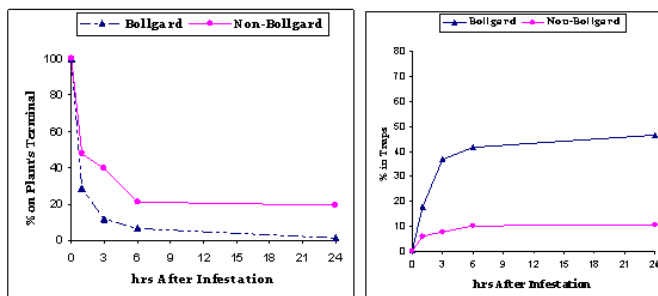


Figure 1 Percentage (% ± standard error) of bollworm larvae survive on plant terminals and recovered from sticky traps for infestations on non-flowering Bollgard® and Non-Bollgard (Conventional) cotton plants

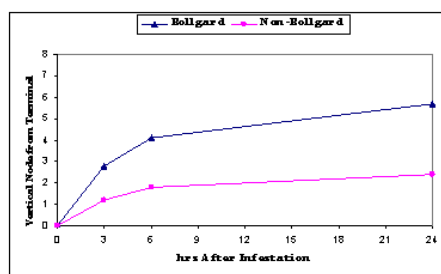


Figure 2 Vertical movement of bollworm larvae from Bollgard® and non-Bollgard (Conventional) cotton plants terminals occurred in the percentage (± standard error)

Helicoverpa armigera Movement on Flowering Cotton Plants

Similar to the results for individual pre-flowering cotton plants, bollworm larvae moved significantly more on individual flowering Bollgard® plants compared to conventional plants. Cotton type ($F=59.67$; $df=1,8$; $P<0.01$), time of evaluation ($F=29.76$; $df=2,16$; $P<0.01$), and the cotton plant type by time of evaluation interaction ($F=5.16$; $df=2,16$; $P=0.02$) was significant for numbers of main stem nodes larvae were found below terminals (Figure 2) Within 3hr, larvae moved 2.8 nodes below the terminal on Bollgard® cotton plant whereas those larvae on conventional cotton plant moved 1.2 nodes below the terminal. *Helicoverpa armigera* larvae were found 4.1 main stem nodes below plant terminals on Bollgard® cotton plant compared to 1.8 main stem nodes below plant terminals on non- Bollgard cotton plant at 6hr. At 24hrs, larvae were found an average of 5.7 main stem nodes below the terminals on Bollgard® plants compared to 2.4 main stem nodes below the terminals on conventional (non- Bollgard) cotton plant.

Cotton plant type ($F=24.20$; $df=1,8$; $P<0.01$) and time of evaluation ($F=9.14$; $df=2,16$; $P<0.01$) effects were significant for numbers of bollworm infested terminals. For numbers of bollworm infested squares ($F=5.59$; $df=2,16$; $P=0.01$) and bolls ($F=5.34$; $df=2,16$; $P=0.02$) there were cotton plant type by time of evaluation interactions (Figure 3). Also, there was a cotton plant type effect for numbers of bollworm infested white flowers ($F=36.42$; $df=1,8$; $P<0.01$). On Bollgard® cotton plants, fewer larvae remained in plant terminals compared to conventional cotton plants at all rating intervals. Fewer larvae were observed on Bollgard® cotton plant squares than on conventional cotton plants squares at 24hr. Consequently, more larvae were recovered lower in the plant canopy in white flowers (1.0vs0.1) and bolls (4.7vs0.8) on Bollgard® cotton plants than on conventional plants at 24hr. No larvae were recovered from conventional white flowers at 3hr and 6hr. In the micro-plots, numbers of plants, squares, flowers, and bolls ranged from 5 to 10, 56 to 153, 0 to 9, and 24 to 87, respectively, (Figure 4) within Bollgard® cotton plant and

conventional cotton plant micro-plots during the infestation period. There was a cotton plant type by time of evaluation interaction for the percentage of bollworm *Helicoverpa armigera* infested terminals ($F=14.78$; $df=1,88$; $P<0.01$). Also, percentages of infested squares ($F=12.09$; $df=1,88$; $P<0.01$), white flowers ($F=14.15$; $df=1,88$; $P<0.01$), and bolls ($F=28.20$; $df=1,88$; $P<0.01$) were different between cotton plant types.

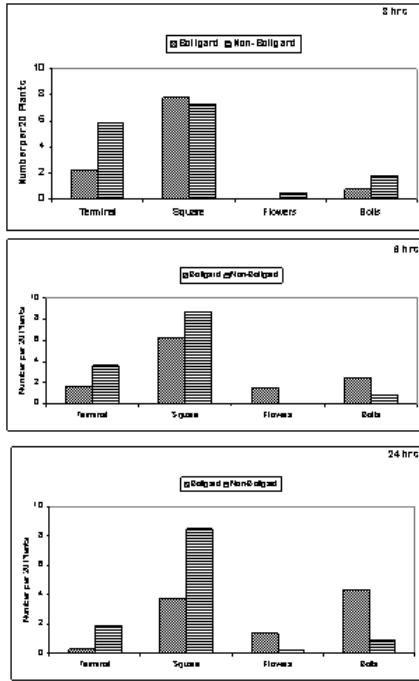


Figure 3 Percentage of bollworm infested plant structures within the canopies of individual flowering Bollgard® and non-Bollgard (Conventional) cotton plants at 3, 6, and 24hrs after infestation (\pm standard error)

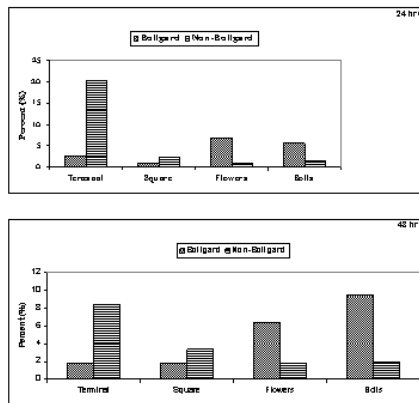


Figure 4 Percentage of infested Bollgard® and non-Bollgard (Conventional Cotton) plant structures within micro-plots at 24 and 48 h after infestation.

Fewer bollworm larvae remained in plant terminals of Bollgard® cotton plants (1.8%) compared to that of conventional cotton plants (20.3%) at 24hr. Within 48hr, the percentage of bollworm infested terminals decreased to 8.6% for conventional cotton plant; however, this remained higher

than for Bollgard® cotton plants (1.5%). Also, the percentage of *Helicoverpa armigera* bollworm infested squares was lower on Bollgard® cotton plant (1.1 to 1.5%) than on conventional cotton plant (2.2 to 3.1%). Similar to the previous experiment, the percentages of infested white flowers and bolls were higher on Bollgard® cotton plant than on conventional cotton plants. On Bollgard® cotton plants, the percentages of bollworm infested white flowers was 8.0% at 24hr and 6.8% at 48hr; whereas, the percentage of bollworm infested white flowers on conventional cotton plants was less than 1.5%. Similarly, the percentage of infested bolls exceeded 7.5% at 24hr and 48hr on Bollgard cotton plants and remained less than 2.0% on conventional cotton plants.

Discussion

Large numbers of *Helicoverpa armigera* bollworm larvae have been observed in white flowers of Bollgard® cotton plant every year since its introduction in 2002. Bollworm *Helicoverpa armigera* eggs are generally concentrated the top one third of cotton plants and the majority of eggs are usually near plant terminals (Wilson et al. 1980, Farrar and Bradley 1985). Small *Helicoverpa armigera*, bollworm larvae remain near the terminals of conventional cotton plants feeding on small squares (Reese et al. 1981). Fye (1972) found that 78 to 100% of damaged fruiting forms could be found in the top 0.6-m of plants at any given time. As larvae develop, they typically move down the plants feeding on larger squares and bolls (Wilson and Gutierrez 1980).

Data in the present study indicate that bollworm *Helicoverpa armigera* larvae disperse more rapidly on Bollgard® cotton plants compared to conventional cotton plants. Bollworm larvae moved 2.9 main stem nodes below Bollgard® cotton plant terminals within 3hr, but only moved 2.5 main stem nodes within 6hr on conventional cotton plants. Also, those larvae ultimately moved a greater vertical distance on Bollgard cotton plants (5.7 nodes at 24hr) than on conventional (non-Bollgard) cotton plants (2.4 nodes at 24hr). Larvae remained near the top of conventional cotton plants feeding on terminal foliage and small squares. In contrast, larvae were observed lower in the plant canopy on Bollgard® cotton plant feeding on white flowers and bolls. Results similar to those found in the present study have been observed previously. Benedict et al. (1992, 1993) and Parker and Luttrell (1999) found that *Helicoverpa virescens* larvae exhibit different dispersal patterns on Bollgard® cotton plant than on conventional cotton plant. In those studies, higher numbers of *Helicoverpa virescens* larvae left the terminals of Bollgard® cotton plant than conventional cotton plant. Bollworm larvae may exhibit this same behaviour because they have demonstrated the ability to detect and avoid *Bacillus thuringiensis* proteins in foliar sprays (Jyoti et al. 1996, Greenplate et al. 1998). In the present study, bollworm larvae began migrating away from Bollgard® cotton plant terminals within 1hr. Within 6hr, less than 10% of larvae remained in Bollgard® cotton plant terminals. In a laboratory bioassay, Gould et al. (1991) found that *Helicoverpa virescens* larvae were able to avoid *Bacillus thuringiensis* proteins. Also, previous studies have shown that *Bacillus thuringiensis* proteins elicit avoidance behaviour in other insects including the light brown apple moth larvae, *Epiphyas postvittana* (Walker), (Harris et al. 1997); gypsy moth larvae, *Lymantria*

dispar (L.), (Yendol et al. 1975); and several insect pests of corn (Mohd-Salleh and Lewis 1982). In addition, bollworm larval behaviour is affected by natural allelochemicals in cotton plant (Schmidt et al. 1988) and tomato (Cosenza and Green 1979, Binder and Bowers 1991, Juvik et al. 1994).

Cotton pest management consultants have experienced difficulties in making decisions about when to apply foliar insecticides to manage bollworms in Bollgard® cotton plant. Currently, action thresholds to initiate cotton bollworm control with foliar sprays are based on numbers of eggs and/or larvae in terminals, and numbers of larval infested/damaged squares on conventional cotton plant. If current thresholds for *Helicoverpa armigera* (Bollworm) and *Helicoverpa virescens* (Tobacco Budworm) conventional cotton plants are used, the assumption that bollworm damage potential is the same on Bollgard® and conventional cotton plant would have to be met. Gore et al. (2002) found that an individual bollworm larva damaged as many as 3.5 fruiting forms on Bollgard® cotton plant compared to 6.6 on conventional cotton plant. Therefore, current thresholds for conventional cotton plant are not appropriate for Bollgard® cotton plant because damage potentials are not the same. Currently, conventional cotton plant fields are scouted by examining plant terminals and squares. Current scouting methods are not appropriate for Bollgard® cotton plant because larvae feeding on white flowers and bolls may be overlooked. For the 1 meter row infestations, the percentage of infested terminals averaged 12.2% on conventional cotton plant at 48hr. This level is above the current action threshold and the conventional plots would be treated with foliar insecticide applications. Also, 3.2% of non-Bollgard squares were infested with larvae. In contrast, 1.2% and 0.8% of Bollgard terminals and squares were infested with larvae, respectively, within 48hr. Based on current action thresholds, Bollgard cotton plant would not require treatment. However, if the percentages of infested flowers (9.7%) and bolls (4.2%) are also considered, Bollgard® cotton plant may require insecticide applications to prevent economic yield loss. In addition, bollworm larvae began moving out of plant terminals within 1hr on Bollgard® cotton plant. Therefore, when eggs hatch, there is a narrow period of time when larvae can still be observed in or near plant terminals. Over 90% of larvae that were originally infested on pre-flowering Bollgard® plants migrated away from plant terminals within 6hrs. Field scouts searching for bollworm infestations in Bollgard® cotton plant are likely not to find larvae in the terminals when sampling more than 6hr after larval eclosion.

These data suggest that current scouting protocols and action levels to initiate insecticide treatments for bollworms on non-Bollgard cotton plant are not appropriate for Bollgard® cotton plant. Scouts should look at white flowers and small bolls in addition to terminals and squares when scouting Bollgard® cotton plant because bollworm larvae migrate to those structures in a relatively short time. This information is necessary to further refine action thresholds for bollworms in Bollgard® cotton plant.

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