In-Field Monitoring of Beneficial Insect Populations in Transgenic Corn Expressing a *Bacillus thuringiensis* Toxin[1]

Suzanne J. Wold, Eric C. Burkness, W. D. Hutchison and Robert C. Venette[2]

Department of Entomology, University of Minnesota and [2]USDA-APHIS, Department of Entomology, 1980 Folwell Ave., St. Paul, Minnesota 55108-6125 USA

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Abstract In 1998 and 1999, field studies were conducted near Rosemount, MN to assess the potential impact of transgenic sweet corn, transformed to express the Cry1Ab toxin from *Bacillus thuringiensis* Berliner var *kurstaki* (i.e., Bt corn), on several beneficial insects, including predatory coccinellids, chrysopids and anthocorids. Beneficial insects in both Bt and in non-Bt sweet corn were also monitored in field cages in 1999. Plants were visually sampled for beneficial insects by arbitrarily selecting 3 consecutive plants from each plot or 6 plants/cage. Rank transformed data were analyzed using the Kruskal-Wallis test, which indicated no significant within-year differences in the overall density of beneficial insect populations between Bt and non-Bt sweet corn. *Coleomegilla maculata* (DeGeer) was the dominant predator species detected in 1998 and 1999. A significant trend ($P < 0.05$) was found for *C. maculata* larvae in open plots, with non-Bt treatments having higher *C. maculata* levels than Bt. Also, *C. maculata* larval and adult densities, for caged plots, showed a significant trend for higher counts in the in non-Bt corn. No additional differences in species diversity of beneficial insects were detected using Hills N1. Neither *Hippodamia convergens* Guérin-Ménville, *Adalia bipunctata* (L.), nor *Coccinella septempunctata* L. were observed during 1999. Although our test detected significant trends for higher densities of *C. maculata* in non-Bt corn, the results also suggest that longer-term in-field studies with higher sample sizes are needed to further characterize what may be relatively subtle population effects in the field.

Key Words Beneficial insects, Coccinellidae, Chrysopidae, predators, Bt sweet corn

The European corn borer, *Ostrinia nubilalis* (Hübner), is a major economic pest of corn, *Zea mays* L. (Mason et al. 1996). Larvae cause damage by feeding on ears or within the stalks, which may result in lodging or ear-drop, and by disrupting plant physiological processes. For sweet corn, consumers are reluctant to accept the presence of *O. nubilalis* larvae or damage in ears, making control of this pest a priority for growers and processors (e.g., Gingera et al. 1993).

Over the past 50 years, several measures have been introduced to control *O. nubilalis,* including importation of natural enemies and the use of insecticides (Mason et al. 1996). More recently, corn hybrids have been genetically altered to express an insecticidal toxin from *Bacillus thuringiensis* Berliner (Bt) subsp. *kurstaki,* Berliner (Ostlie et al. 1997). Specifically, for transgenic sweet corn hybrids, Bt toxins are expressed in green leaf tissue as well as pollen, silk and kernel tissue (Lynch et al.

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1999). For most Bt corn hybrids, production of toxin continues until plant senescence at which time toxin levels begin to decline (Armstrong et al. 1995).

The potential economic benefits provided by transgenic insecticidal crops are well known (Ostlie et al. 1997, Rice and Pilcher 1998, Burkness et al. 2001), but concerns have arisen regarding the possibility of insects developing resistance to the toxin (Tabashnik 1994, Bolin et al. 1999). More recently, new concerns have been raised regarding the potential direct or indirect effects that Bt may have on populations of natural enemies of corn pests (Orr and Landis 1997, Hilbeck et al. 1998b), such as predatory coccinellids. Several generalist predators are known to cause significant *O. nubilalis* mortality (e.g., Andow 1990). These predatory species are found in corn for several reasons: pollen is a food source, plants provide a substrate for oviposition, and plants serve as a reservoir for insect prey and hosts including *O. nubilalis* (Hodek 1973, Andow 1990, Coll and Bottrell 1991). Only recently, have studies dealt with the possible adverse effects that transgenic Bt toxins may have against beneficial insect populations in field corn (e.g., Pilcher et al. 1997, Orr and Landis 1997, Hilbeck et al. 1998a), and to date none have been conducted in sweet corn. Furthermore, many of the past studies dealing with the effects of Bt on predaceous insects have dealt with the commercially available foliar applied Bt products (e.g., Flexner et al. 1986). Because transgenic Bt toxins are expressed in a modified, truncated form different than microbial insecticides (Fietelson et al. 1992, Koziel et al. 1993), it may not be appropriate to determine non-target effects from the results of foliar applied Bt experiments (Jepson et al. 1994).

Both direct and indirect effects are of particular concern for beneficial insects. Predators such as the coccinellid, *Coleomegilla maculata* (DeGeer), and the green lacewing, *Chrysoperla carnea* (Stephens), may be affected. *Coleomegilla maculata* feeds upon *O. nubilalis* eggs (Andow 1990), aphids (Smith 1965), and pollen, which can make up 50% of its diet (Hodek 1993), while *C. carnea* feeds upon pollen and various insect pest species (Obrycki et al. 1989). In a recent study, Hilbeck et al. (1998b) documented the first direct mortality effect of Bt toxin on *C. carnea* larvae. In contrast, non-target insect pests could feed upon the transgenic plant, ingest the Bt toxin, and pass the toxin to a predatory insect (Hilbeck et al. 1998a). Both studies indicate that Bt has adverse effects on *C. carnea* larvae in the laboratory setting, yet it is not certain how these effects will transfer to the field environment. The objectives of this study were to (1) document beneficial insect species diversity in Minnesota sweet corn, primarily from tassel to harvest and (2) to measure potential effects of transgenic Bt sweet corn on populations of the insects identified.

Materials and Methods

1998 and 1999 open field plots. The Bt sweet corn hybrid 'GH-0937', which expresses Cry1Ab, and its non-Bt isoline 'Bonus' were planted in field plots in 1998 and 1999 at the Rosemount Experiment Station, Rosemount, MN. In 1998, sweet corn was planted on 1 June (early) and 26 June (late). Plots were 4 rows wide with 76.2 cm row spacing and 9.14 m in length. Treatments were arranged in a randomized complete block design with 4 replications. In 1999, sweet corn was planted on 9 June and 15 June; plots were larger, at 30 rows wide with 76.2 cm row spacing, and 24.6 m in length. Treatments were arranged in a randomized complete block design with 4 replications.

Throughout this study, the sample unit consisted of 3 consecutive plants, visually

inspected for all predator species (adults and immatures). Visual sampling consisted of the arbitrary selection of three consecutive plants from the interior of the plot for identification and counts of predatory insects. The silk, tassel, leaves, stalk and tillers were examined for both adult and immature stages of beneficial insects. Predator identification and count data were recorded in the field. Coccinellid species were identified according to voucher specimens provided by Schellhorn (1998) and a diagnostic guide (Schellhorn 2000). Identification of other predator and pest species were confirmed based on voucher specimens in the Insect Museum, Department of Entomology, University of Minnesota.

In 1998, visual sampling was conducted for three sample dates at both the whorl (before anthesis) and silk (during anthesis) growth stages, for two planting dates. Thus, for each planting date, 6 samples were taken for a total of 12. Sampling began in the early-planted whorl stage sweet corn on 24 July and continued until 7 August. Silk stage monitoring in the early-planted sweet corn began 17 August. This date coincided with the first visual sample for the whorl stage of the late-planted sweet corn. Sampling in late-planted sweet corn at silk stage began on 31 August and ended 14 September, at which time plots were harvested.

In 1999, sampling was conducted weekly throughout the silk stage (from anthesis to brown silk) of the plants for a total of 3 sample dates, specifically 17, 24, and 31 August. Plants were visually sampled for beneficial insect species and aphids. The number of beneficial species was recorded as well as the number of individuals in each species.

1999 caged plots. The Bt sweet corn hybrid 'GH-0937', and its non-Bt isoline 'Bonus' were planted 25 June. Plots were 8 rows wide with 76.2 cm row spacing and 9.14 m in length. Two cages (1.82 × 1.82 × 2.74 m, 32 × 32 cm Lumite screen, BioQuip Inc.) were placed in the interior of each plot, for a total of 16 cages. Each cage contained 16 plants. In addition to the caged plots, we sampled on either side of the cage, leaving 2 border rows to the outside and to the cage.

Sampling was done twice per week beginning 31 August and ending 16 September, for a total of 6 sample dates, from tassel to brown silk. Two sets of 3 consecutive plants each were sampled in each cage and in each adjacent open plot. Whole plant samples were conducted as described previously. Both beneficial insects and aphids were counted. To supplement the beneficial insect population, 8 *C. maculata* adults that were collected from a neighboring non-Bt field corn plot and added to each of 4 cages placed over Bt and non-Bt sweet corn.

Data analysis. All data were analyzed using non-parametric statistics; specifically, all data were sorted in ascending order and ranked. The Kruskal-Wallis test (Conover and Iman 1981) was used to test for differences between the mean ranking of each treatment. Non-parametric statistics were chosen because variances were found to not be homogeneous ($P < 0.05$) using Lavene's test (Lavene 1960), and transformation of the data did not rectify the situation. In 1998 and 1999, data were compared by type of corn (Bt and non-Bt) and sample date. Due to the lack of significant differences between sample date in 1998 and 1999, data were pooled within each year. Data from 1998 were also compared by planting date and growth stage (data not presented). All life stages of insects were recorded during each sampling period for the open plots. However, because of the possibility of adult movement between plots, only immature stages were analyzed.

Both adult and immature stages of the insects were used in the analyses of the cage study. Comparison between infested and non-infested cages for Bt and non-Bt

treatments showed no significant differences. Therefore, data were combined for the infested and non-infested cage treatments.

Shannon's index was used to calculate Hill's N1, which measures the number of abundant species in a sample (Ludwig and Reynolds 1988).

Results

1998 and 1999 open field plots. During both years, immature stages of 7 predatory insect species were detected: the 12-spotted lady beetle, *C. maculata;* the 13-spotted lady beetle, *Hippodamia tredecimpunctata tibialis* (Say); the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas); the 2-spotted lady beetle, *Adalia bipunctata* (L.); the insidious flower bug, *Orius insidiosus* (Say); the common damsel bug, *Nabis americoferus* Caryon, and the green lacewing, *Chrysoperla carnea* (Stephens). Two additional species were detected in 1998, including the convergent ladybeetle, *Hippodamia convergens* Guérin-Ménville, and the 7-spotted ladybeetle, *Coccinella septempunctata* L.

We detected few differences in beneficial insect populations between Bt and non-Bt sweet corn for 1998 or 1999 (Table 1). The average number of species per plant and number of abundant species (measured by Hill's N1) did not differ between Bt and non-Bt sweet corn hybrids. In both years, across all sample dates, *C. maculata* was the dominant species in the beneficial insect community, on average representing 43% of the beneficial insects in the Bt sweet corn and 51% in the non-Bt sweet corn. *Chrysoperla carnea* was generally scarce, often averaging <2% of the entire population (Table 1).

In 1998, *C. maculata* accounted for 43% of beneficial insects observed in the Bt plots and 56% in non-Bt (Table 1). In addition, *C. maculata* was equally common during the whorl and silk stage, comprising 50 and 47% of the beneficial species at each growth stage, respectively. Neither *C. septempunctata* nor *C. carnea* were detected during the silk stage (Table 1). During the silk stage, both *A. bipunctata* and *N. americoferus* were observed in the Bt plots at low levels, yet absent in non-Bt plots.

In 1999, no differences were found between beneficial insect populations in Bt and non-Bt sweet corn with the exception of *C. maculata* (Table 1). Numbers of *C. maculata* were significantly lower in Bt plots than in non-Bt plots. Both *C. maculata* and *O. insidiosus* were the most abundant species in both Bt and non-Bt plots, with *C. maculata* representing 39% and 50% of predatory individuals in Bt and non-Bt sweet corn, respectively; *O. insidiosus* represented 33 and 37% in Bt and non-Bt sweet corn, respectively (Table 1). Neither *H. convergens* nor *C. septempunctata* were observed during the 1999 samples. Both *C. carnea* and *N. americoferus* were only observed in the non-Bt sweet corn. Aphid populations, primarily corn leaf aphid, *Rhopalosiphum maidis* (Fitch), decreased throughout the sample period, averaging 2.67 and 1.92 aphids/3 plants in the Bt and non-Bt plots, respectively. No significant differences were evident in aphid population density between Bt and non-Bt plants ($P = 0.52$).

1999 caged field plots. Adult and immature *C. maculata, H. tredecimpunctata tibialis, O. insidiosus, H. axyridis, C. carnea* and *N. americoferus* were detected inside the cages. Only *C. maculata, H. t. tibialis, H. axyridis,* and *C. carnea* were found in corn rows immediately outside the cages (Table 2).

Rank transformation tests indicated, for a majority of the sample dates, a significant trend for higher densities of *C. maculata* larvae and adults on non-Bt corn inside

Table 1. Mean density of beneficial insects per 3 plants in open plots, 1998-1999, Rosemount, Minn.

Species	1998								1999			
	Whorl				Silk				Silk			
	Bt	Non-Bt	*n**	χ^2; *P***	Bt	Non-Bt	*n*	χ^2; *P*	Bt	Non-Bt	*n*	χ^2; *P*
Coleomegilla maculata	1.54	1.71	6	0.00; 1.00	0.88	1.29	6	0.00; 1.00	1.17	1.92	6	4.89; 0.03
Hippodamia convergens	0.04	0.04	6	0.00; 1.00	0.00	0.04	6	1.00; 0.32	0.00	0.00	6	0.00; 1.00
H. t. tibialis	0.50	0.42	6	1.38; 0.24	0.13	0.17	6	0.41; 0.52	0.17	0.13	6	1.00; 0.32
Coccinella septempunctata	0.08	0.25	6	1.00; 0.32	0.00	0.00	6	0.00; 1.00	0.00	0.00	6	0.00; 1.00
Adalia bipunctata	0.42	0.25	6	2.20; 0.14	0.04	0.00	6	1.00; 0.32	0.04	0.13	6	0.00; 1.00
Harmonia axyridis	0.29	0.25	6	1.00; 0.32	0.21	0.25	6	1.00; 0.32	0.63	0.17	6	2.83; 0.09
Orius insidiosus	0.50	0.04	6	1.38; 0.24	0.54	1.04	6	1.38; 0.24	1.00	1.42	6	0.00; 1.00
Chrysoperla carnea	0.08	0.04	6	1.00; 0.32	0.00	0.00	6	0.00; 1.00	0.00	0.04	6	0.00; 1.00
Nabis americoferus	0.17	0.08	6	2.20; 0.14	0.04	0.00	6	1.00; 0.32	0.00	0.04	6	0.00; 1.00
Sum of species+	1.74	1.71	6	0.00; 1.00	1.67	1.62	6	0.31; 0.58	1.70	1.60	6	0.31; 0.58
Hill's N1++	1.67	2.75	6	1.22; 0.27	1.63	1.54	6	1.22; 0.27	1.67	1.67	6	0.00; 1.00

* Number of sample dates used to calculate χ^2 values.

** χ^2 values significantly different if $P \leq 0.05$.

\+ Refers to average number of species observed per plot over a given period. All species were not represented in each plot on each sample date.

++ Average number of abundant species observed per plot (Ludwig and Reynolds 1988).

Table 2. Mean density of beneficial insects per 3 plants in caged and open plots, 1999, Rosemount, Minn.

	Plots inside cages								Open plots adjacent to cages			
	Immatures (mean no./3 plants)				Adults (mean no./3 plants)				Immatures (mean no./3 plants)			
Species	Bt	Non-Bt	n*	χ^2; P**	Bt	Non-Bt	n	χ^2; P	Bt	Non-Bt	N	χ^2; P
Coleomegilla maculata	1.05	1.21	6	1.22; 0.27	0.77	1.00	6	1.22; 0.27	1.48	1.38	6	0.00; 1.00
Hippodamia t. tibialis	0.01	0.00	6	0.00; 1.00	0.01	0.00	6	1.00; 0.32	0.04	0.10	6	2.20; 0.14
Harmonia axyridis	0.18	0.15	6	0.00; 1.00	0.15	0.13	6	0.31; 0.58	0.00	0.02	6	1.00; 0.40
Orius insidiosus	0.06	0.02	6	0.41; 0.52	2.10	2.35	6	1.00; 0.32	2.48	2.33	6	1.22; 1.27
Chrysoperla carnea	0.03	0.00	6	2.20; 0.14	0.00	0.01	6	1.22; 0.27	0.00	0.00	6	0.00; 1.00
Nabis americoferus	0.00	0.00	6	0.00; 1.00	0.96	1.14	6	2.83; 0.93	0.00	0.00	6	1.00; 0.32
Sum of species+	1.74	1.88	6	1.22; 0.27	1.96	1.65	6	1.38; 0.24	1.27	1.44	6	1.38; 0.24
Hill's N1++	1.61	1.64	6	1.22; 0.27	1.71	1.69	6	0.00; 1.00	1.58	1.73	6	4.89; 0.03

* Number of sample dates used to calculate χ^2 values.

** χ^2 values significantly different at $P \leq 0.05$.

+ Refers to average number of different species observed per plot over a given sample period. All species were not represented in each plot on each sample date.

++ Average number of abundant species observed per plot (Ludwig and Reynolds 1988).

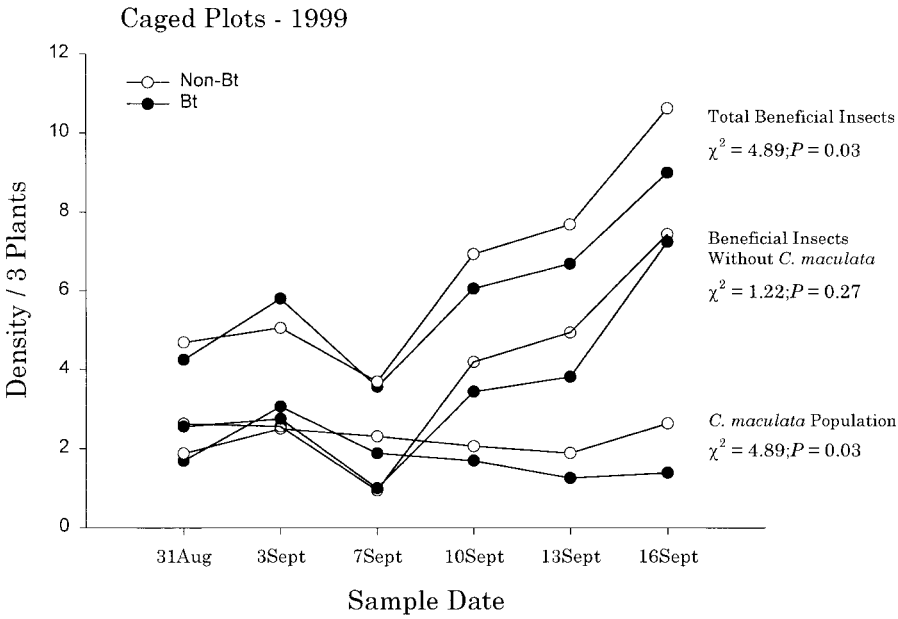

Fig. 1. Density of *C. maculata* and other beneficial species (adults and immatures combined) on Bt and non-Bt sweet corn within field cages (Kruskal-Wallis χ^2 provides non-parametric test of differences between Bt and non-Bt across sampling periods; see text).

the cages, (χ^2 = 4.89, P = 0.03) (Fig. 1), and for the total predator population (χ^2 = 4.89, P = 0.03) (Fig. 1). In corn plots adjacent to the cages more abundant species were found on the non-Bt corn than the Bt corn (χ^2 = 4.89, P = 0.03) (Table 2). In corn plots adjacent to the cages, the most abundant immature species was *O. insidiosus,* averaging 62% of the total beneficials observed in the Bt and 61% in non-Bt sweet corn (Table 2). Within the cages the most abundant immature species observed was *C. maculata,* averaging 79% in the Bt and 88% in the non-Bt (Table 2).

No differences in the aphid population (*R. maidis*) density, between Bt and non-Bt corn, were detected in caged plots or plots adjacent to the cages. Over 6 sample dates, there were 0.33 aphids/3 plants in Bt caged plots and 0.17 aphids/3 plants in non-Bt caged plots (P = 0.17). Similarly, plots adjacent to the cages harbored low densities with a season average of 0.71 aphids/3 plants in Bt and 0.13 aphids/3 plants in non-Bt (P = 0.18).

Discussion

To limit possible confounding effects from adult insect movement in and out of open Bt and non-Bt plots, we used only data for immature stages of each beneficial species. Most of the beneficial insects studied, especially coccinellids, are highly mobile as adults (Edwards 1986). In 1999, we addressed this concern by conducting

the research in caged plots, and therefore used both the immature and adult data for our analysis.

No significant differences were detected between Bt and non-Bt sweet corn for total predator density, or species diversity, of immature beneficial insects in the 1998 or 1999 open plots. However, across sample dates, higher levels of *C. maculata* were found in non-Bt sweet corn in 1999, in both open plots and caged plots (Table 2, Fig. 1). Total beneficial insects also tend to be higher in the non-Bt caged plots (Fig. 1). However, when *C. maculata* was removed from the total predator population, the trend disappears, suggesting that the difference was likely due to *C. maculata*. In 1999, *C. maculata* density was significantly higher in the non-Bt sweet corn (silking stage) (Table 1). In 1998 and 1999, the dominant beneficial insect species detected in open plots were *C. maculata* and *O. insidiosous* (Table 1). These results are also similar to those found in Minnesota sweet corn during a 1991-1992 study (Bolin et al. 1996). *Coleomegilla maculata* is polyphagous and feeds on aphids, pollen, *O. nubilalis* eggs and other pest eggs. Additionally, *C. maculata* populations are known to increase in response to aphid populations (Wright and Laing 1980), which may have been present during the whorl stage of our study (Foott 1977). All of these factors may have contributed to the overall population density observed in our study.

In a similar study, Pilcher et al. (1997) did not find any statistical differences in beneficial insects monitored in Bt and non-Bt field corn. However, factors other than overall population levels should be considered before concluding that Bt sweet corn has no adverse affects on beneficial insects. For example, Hilbeck et al. (1998b) found that *C. carnea* larvae fed on a diet containing Cry1Ab toxin had significantly greater mortality (57%) than those not fed the toxin (30%). To explore possible indirect effects of Bt toxin, *C. carnea* larvae were fed a prey species (*Spodoptera littoralis* (Boisduval), relatively insensitive to Bt) that had been reared on a diet containing Cry1Ab toxin (Hilbeck 1998a). Mortality of *C. carnea* was significantly greater (62%) when reared upon prey that were fed Bt diet than those that were not fed the diet (37%).

In both caged plots and the plots adjacent to the cages, no differences were observed between the majority of beneficial insect populations in Bt and non-Bt sweet corn. However, in caged plots *C. maculata* was generally more abundant in the non-Bt sweet corn. This suggests that Bt corn may have an adverse effect on *C. maculata* and that more extensive sampling may be necessary to observe an effect from Bt in larger plots. Direct laboratory bioassays with Bt corn, or Bt pollen, and *C. maculata* are also warranted.

Preservation of beneficial insects such as coccinellids is an important strategy in integrated pest management (e.g., Mason et al. 1996). Utilization of transgenic Bt sweet corn may in fact prove to be an effective and environmentally benign alternative for growers (Rice and Pilcher 1998). Transgenic Bt sweet corn has the potential to virtually eliminate the need for insecticidal sprays (Lynch et al. 1999, Burkness et al. 2001) that may be harmful to beneficial insects. Consequently, continued studies of the interactions between natural enemies and transgenic plants, and systematic monitoring of beneficial species, are recommended for the long-term management and sustainability of Bt corn.

In summary, few statistical differences and inconsistent numerical trends might suggest that Bt has no adverse effects on beneficial insects in the field. However, field experiments in this study were designed to detect large differences among insect populations in Bt and non-Bt corn. With field cage studies, the non-parametric analy-

sis allowed us to detect differences in the number of sample dates with higher *C. maculata* counts in non-Bt corn. This analysis, however, does not allow for testing the magnitude of those differences. The ability to detect differences depends on the number of replications, the density of insect populations, and variances associated with population estimates (Zar 1996). Although the use of 4 to 8 replications in our study is typical of field experiments (e.g., Pilcher et al. 1997), the density of predators was relatively low and the associated variance was high. Consequently, any effects of Bt corn on predators may simply be smaller than we could detect with conventional replications and sample size. Thus, predator population effects with transgenic corn will likely require higher sample sizes and may be important over large spatial scales or periods of time, all of which warrant further investigation.

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