Temporal and spatial overlap between monarch larvae and corn pollen

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To assess the likelihood that monarch larvae will be exposed to *Bacillus thuringiensis* (Bt) pollen, we studied milkweed and monarch densities in habitats which comprise much of the land available to breeding monarchs, e.g., cornfields, cornfield edges, other agricultural fields, and nonagricultural areas, in four regions of the monarch breeding range. We found that monarchs use milkweed in cornfields throughout their breeding season, and that per plant densities are as high or higher in agricultural habitats as in nonagricultural habitats. As a result of the prevalence of agricultural land, most of the monarchs produced in the upper Midwest are likely to originate in cornfields or other agricultural habitats. There was a greater temporal overlap between susceptible monarchs and corn anthesis in the northern than the southern part of the summer breeding range, because of earlier pollen shed in the south. The importance of agricultural habitats to monarch production suggests that, regardless of the impact of genetically modified crops, agricultural practices such as weed control and foliar insecticide use could have large impacts on monarch populations.

Transgenic *Bacillus thuringiensis* (Bt) corn (*Zea mays* L.) plants were designed to control the European corn borer [*Ostrinia nubilalis* (Hübner)] and other Lepidoptera feeding on corn tissue. The Bt toxins produced by transgenic corn are lepidopteran-specific and kill only insects that ingest the plant tissue. Because of this specificity, the impact of Bt corn on nontarget organisms was assumed to be negligible (1–3). However, most commercial Bt hybrids express the endotoxin in their pollen to varying degrees and thus may impose risks to nontarget Lepidoptera that consume pollen deposited on their host plants (4, 5).

Many aspects of monarch butterfly (*Danaus plexippus* L.) biology may make them particularly susceptible to impacts of corn pollen exposure. Monarch larvae are present in the late summer, when a portion of the corn acreage is pollinating. A recent stable isotope study (6) suggested the Corn Belt is the origin of most monarchs that migrate to Mexico and form the nucleus of the following year's population. Malcolm *et al.* (7) estimated that 92% of the monarchs overwintering in Mexico had fed as larvae on *Asclepias syriaca* (common milkweed), a common weed in agricultural habitats (8–10).

To determine the potential impact of Bt corn on monarch populations, we need to know the proportion of the monarch population that overlaps temporally and spatially with corn pollen during the larva stage. To estimate this quantity, we measured relative monarch densities in different habitats and the temporal overlap between monarch larvae and corn anthesis. In addition, we compared survivorship of larvae in different habitats to determine whether oviposition patterns reflect monarch production from each habitat.

Materials and Methods

Study Sites. The study was conducted in four regions representing different parts of the breeding range of eastern North American monarchs: (*i*) east central Minnesota and west central Wisconsin, (*ii*) central Iowa, (*iii*) coastal Maryland, and (*iv*) southern Ontario.

Research groups in each region monitored five sites where different habitat types were represented (Table 1). All sites included a field planted in nontransgenic corn (except Maryland and Iowa, where one site contained transgenic corn) and most included a nonagricultural area. Nonagricultural areas were neither planted in crops nor highly urban, and included old fields, restored prairies, or pastures. In addition, Minnesota/Wisconsin, Iowa, and Maryland sites included 10-m belts at the edge of cornfields (encompassing roadsides and fencerows), and Iowa and Maryland sites included agricultural fields other than corn. All habitats at a given site were within 1 km of each other to increase the chance that the same monarch population was exposed to all habitats within a site. Only cornfields containing at least 10 milkweed ramets (aboveground stems) per hectare were included to ensure sufficient opportunity to observe monarchs.

Weekly Monitoring of Monarch Abundance. The monitoring procedure was adapted from the Monarch Larval Monitoring Project, a volunteer program initiated in 1997 (ref. 11; www.monarchlab.umn.edu). We monitored U.S. sites weekly from late May or early June through August, when monarchs were present in each location, and the Ontario site in July and August. In each habitat, we searched a large number of milkweed ramets and recorded monarch egg and larval presence on a per ramet basis. In most habitats, we examined ramets along randomly selected belt transects or monitored all ramets in smaller plots within the habitat. In habitats where milkweed density was too low to encounter 200 ramets on transects, we sampled all ramets. The number of ramets sampled per week on transects ranged from 200–1,400, and in complete samples from 25–600. Instars were differentiated by using head-capsule size and tentacle length (12). The frequency and specificity (identifying larvae to instar) of our protocol meant that few individuals could be counted twice in the same stage; at normal summer temperatures, larvae hatch 4–6 days after oviposition, and individual stadia last from 2 to 5 days (13).

All research teams noted the weeks in which over 20% of corn plants were shedding pollen at each site and recorded the height of a random sample of 30 milkweed ramets in each habitat each week. In Iowa, Minnesota/Wisconsin, and Ontario, we measured heights of 10–30 corn plants at each site weekly.

Estimating Milkweed Density. Minnesota/Wisconsin and Ontario research teams quantified milkweed density (in ramets per m²) at each study site by sampling a minimum of 50 m² quadrats along randomly selected transects across the fields in corn and nonagricultural areas (transect lengths varied with the size of the

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Table 1. Site characteristics

Region	Site name	Cornfield size, acres	Anthesis dates (>20% of corn-shedding pollen)	Habitats surveyed	Coordinates
Minnesota/	Farm	40	July 31–August 7	Corn, edge, nonagricultural	45.1N, 92.5W
Wisconsin	Amery	11	July 27–August 3	Corn, edge, nonagricultural	45.3N, 92.4W
	AG	170	July 25–August 1	Corn, edge, nonagricultural	45.1N, 92.5W
	Rosemount	23	July 21–28	Corn, edge, nonagricultural	44.7N, 93.1W
	Cedar Creek	1	July 19–26	Corn*, edge, nonagricultural	45.3N, 93.2W
Iowa	595/280	10	July 7–13	Corn, edge, soybean	41.9N, 93.5W
	Shipley S	180	July 7–13	Corn†, edge, soybean	42.0N, 93.5W
	LincolnWay	80	July 11–17	Corn, edge, nonagricultural	42.0N, 93.7W
	Coopers	40	July 5–19	Corn, edge, soybean	42.0N, 93.7W
	Ankeny	30	July 6–12	Corn, edge, soybean, nonagricultural	41.7N, 93.6W
Ontario	Fergus	38	August 7–14	Corn, nonagricultural	43.4N, 80.2W
	New Hamburg	50	August 1–15	Corn, nonagricultural	43.2N, 80.4W
	Drayton	45	August 10–17	Corn, nonagricultural	43.5N, 80.2W
	Linwood	35	August 9–16	Corn, nonagricultural	43.5N, 80.3W
	Palmerston	100	August 4–18	Corn, nonagricultural	43.5N, 80.4W
Maryland	Wye Farm	45	July 19–31	Corn, edge, soybean, nonagricultural	38.5N, 76.1W
	Wye Island	33	July 6–17	Corn, edge, soybean, nonagricultural	38.5N, 76.1W
	Gannon	22	July 7–19	Corn, edge, soybean, nonagricultural	39.1N, 76.5W
	Airport	20	July 27–Aug 9	Corn†, edge, orchard, nonagricultural	39.1N, 76.5W
	Beaverdam	12	July 6–18	Corn, edge, forage crop, nonagricultural	39.0N, 76.6W

*Sweet corn.
†Bt corn (event 176 in Maryland and Bt 11 in Iowa).

field), and a minimum of 30 quadrats in the smaller edge areas (14). In Maryland, we counted the total number of milkweeds at each study site and measured the area of the site by using aerial maps to determine milkweed densities.

Because our study sites were not necessarily representative of available habitats, we obtained landscape milkweed densities when possible. The Ontario team surveyed agricultural and nonagricultural sites in Huron, Kent, and Wellington counties, with 8 replicates of corn and 10 of nonagricultural areas in each county. Four transects (2 × 100-m) within each field were surveyed to determine the number of milkweed ramets per m². The dimensions of each of site were at least 100 × 100 m. Fields were chosen by calling growers before the survey, avoiding bias for fields with high or low milkweed densities. The first nonagricultural habitats observed near the agricultural fields were surveyed, as long as they met dimension and composition criteria (freely growing open field). We used estimates of milkweed densities in Iowa from Hartzler and Buhler (10) for Midwestern study sites. Milkweed density in each habitat type was converted to the proportion of milkweed on the landscape by using data compiled by Taylor and Shields (15) for U.S. sites, and 1983 and 1984 land cover information (the latest dates for which data were available) from the Ontario Ministry of Agriculture, Food, and Rural Affairs (16, 17) for Canadian sites.

Estimating Monarch Production from Each Habitat. To estimate the proportion of monarch production that comes from cornfields, we multiplied three quantities: (*i*) relative monarch production per milkweed ramet in each habitat, (*ii*) relative milkweed ramet density in each habitat, and (*iii*) the proportion of the landscape composed of each habitat type. This product estimates the relative number of monarchs produced in each habitat type, and thus the proportion of monarchs produced in cornfields.

Phenological Overlap. We calculated the percentage of larvae present during corn anthesis by summing per plant densities of first and second instars observed in each cornfield during anthesis and dividing them by the sums of the per plant densities observed during the entire monitoring period. This calculation was done separately for each field, because anthesis dates within a region varied. We used densities rather than counts because the number of milkweed ramets searched varied during the moni-

toring period, and using counts would overrepresent dates on which more plants were searched. We included only first and second instars to avoid counting individuals more than once, as they are likely to still be larvae the following week.

Statistical Analyses. We used repeated-measure ANOVAs to compare monarch use of different habitat types. Separate ANOVAs were carried out for each region, with the different sites within a region acting as replicates. The response variable was the number of eggs per milkweed ramet (arcsine transformed to normalize data), with repeated measures by date. Habitat type, site, and date were main effects. The error term for habitat and site main effects was the habitat × site interaction, and the error term for the date and date × habitat effects was the habitat × site × date interaction.

We calculated survival probability from the proportions of each instar observed relative to egg numbers in each habitat, using the Weibull frequency distribution to analyze survivorship data (18, 19). This analysis allows statistically and ecologically meaningful inferences from model parameters. The form of the model is $S_P(t) = \exp[-t/b)^c]$, $t, b, c > 0$, where $S_P(t)$ is the probability of an individual surviving to age t, b is a scaling parameter that indicates mortality (high b corresponds to low mortality), and c is a shape parameter ($c = 1$ corresponds to constant mortality and $c < 1$ corresponds to decreased mortality with age). Cumulative degree days needed to reach the end of each stage (13) were used for age intervals. We compared parameters within each region by using Welch's unpaired t test with a Bonferroni adjustment for multiple comparisons. Because pupae are rarely observed, and adults can leave their natal area within a few hours of eclosion, we could measure only survival during the larva period. Thus, our method assumes that survival after the larval stage does not vary among habitats.

Results

Egg Densities on Milkweeds in Different Habitats. Monarchs oviposited on milkweed in all habitats throughout the summer (Fig. 1) and were consistently present in cornfields, even in late summer when milkweed ramets were much shorter than the surrounding corn (Fig. 2). Statistical analyses of the effects of habitat type and other variables on monarch densities are summarized in Table 2 (Table 4 summarizes per ramet egg densities over the entire summer).
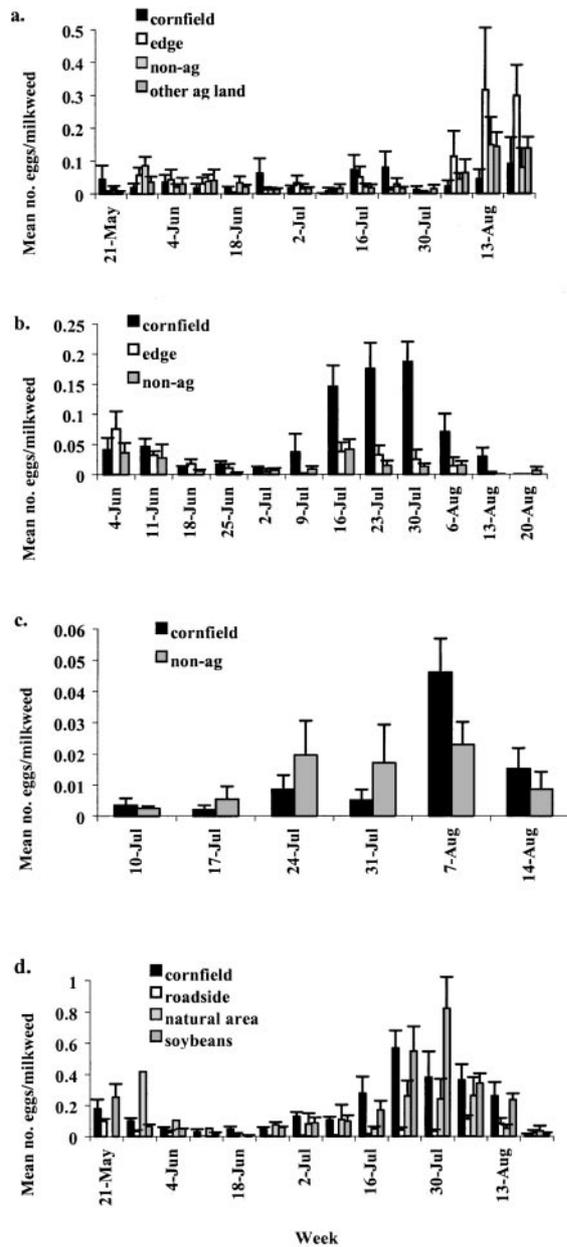
Fig. 1. Average egg densities over time for (*a*) Maryland, (*b*) Minnesota/Wisconsin, (*c*) Ontario, and (*d*) Iowa. Bars represent standard error. Date is the first day of the week in which data were collected. Note that *y* axis scale varies between regions.

Relative egg densities among different habitat types varied by location. In Maryland, where we compared corn, corn edge, and other agricultural and nonagricultural sites, there were no significant differences among habitat types. In Minnesota/Wisconsin, where we compared corn, corn edge, and nonagricultural habitats, there were significantly higher egg densities in cornfields than the other two habitats. In Ontario, where we compared cornfields and nearby nonagricultural sites, there were no significant differences in egg densities between the two habitat types. In Iowa, milkweed plants in agricultural areas (both corn and soybean fields) had higher egg densities than those in cornfield edges. Only two Iowa sites included a nonagricultural habitat, and a separate analysis of these two sites showed no habitat effects ($P > 0.31$; data not shown). Significant date × habitat interactions occurred in Minnesota/Wisconsin and Iowa, where relative egg densities in agricultural
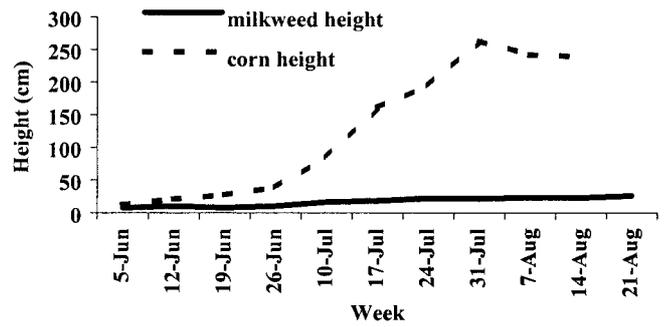

Fig. 2. Average height of milkweed and corn plants for one Minnesota/Wisconsin site. The pattern was similar in all regions, with corn at least twice the height of milkweed by early July.

habitats were higher late in the summer. The significant main effect of date at all sites results from separate peaks in monarch abundance (Fig. 1). There were no significant main effects of site in any region except Maryland, where only two sites differed from each other.

There was no correlation between per ramet egg densities and milkweed densities in surveyed Ontario and Minnesota/Wisconsin fields (data not shown).

Larval Survival in Different Habitats. Fig. 3 illustrates the relative numbers of each instar found in each habitat type over the entire summer, calculated as a proportion of the number of eggs observed. There were significant differences in the *b* parameter in the Weibull model (mortality rate) among habitats in all regions except Maryland (Table 3). In Minnesota/Wisconsin and Ontario, survival was higher in cornfields than other habitats; and in Iowa, survival was higher in corn and nonagricultural habitats than in edge habitats. There were no differences among habitats in the *c* parameter; in all regions and habitats there was higher mortality in the earlier stages ($c < 1$). Despite mortality differences early in development, by the later stadia survival was similar across habitats (Fig. 3). The slight increase at the end of the survival curves in some regions may be the result of smaller sample sizes of the later stadia or higher apparency of fifth instar larvae.

Relative Contribution of Different Habitats to Monarch Production. Calculations of the relative contributions of different habitats are summarized in Table 4. For each habitat within a region, we used the number of eggs observed throughout the season divided by the total number of plants observed to represent relative per ramet contributions to monarch production. We used egg densities rather than later-life stages because the higher sample sizes make these estimates of relative abundance most accurate, and because there were no habitat effects on survival to the later stadia. Milkweed density was generally higher in the nonagricultural habitats than cornfields in all regions (Table 4). This was true in both the monitored fields (Table 4, column 4) and other fields (Table 4, column 5). Because we had no estimates of milkweed density on a landscape basis for Maryland, as we did for Ontario and the upper Midwest, we did not estimate relative monarch productivity in Maryland. We had to survey over 30 fields in Maryland to find 5 that contained high enough milkweed densities to monitor, and are confident that densities in surveyed fields are higher than those in most Maryland cornfields.

Productivity on a per area basis, relative to nonagricultural areas, is shown in column 6, and relative contribution to the total population (taking both overall area and density into account) is shown in column 8 of Table 4. Estimates of productivity suggest that nonagricultural habitats in Ontario are ≈2 orders of magnitude more productive than cornfields on a per area basis. Nonagricul-

Table 2. ANOVA of factors affecting egg densities

Region	Source	df	Sum of squares	F	P
Maryland	Habitat	3	5.54×10^{-2}	1.96	0.174
	Site (replicate)	4	3.25×10^{-1}	8.61	0.002*
	Habitat $\times$ site	12	1.13×10^{-1}		
	Date	6	4.01×10^{-1}	10.62	0.000
	Date $\times$ habitat	18	2.14×10^{-1}	1.86	0.029†
	Habitat $\times$ site $\times$ date	95	5.98×10^{-1}		
Minnesota/Wisconsin	Habitat	2	1.42×10^{-1}	28.68	0.002‡
	Site (replicate)	4	1.89×10^{-2}	1.91	0.202
	Habitat $\times$ site	8	1.98×10^{-2}		
	Date	6	9.81×10^{-2}	9.96	0.000
	Date $\times$ habitat	12	9.03×10^{-2}	4.58	0.000†
	Habitat $\times$ site $\times$ date	70	1.15×10^{-1}		
Ontario	Habitat	1	8.31×10^{-6}	0.02	0.892
	Site (replicate)	4	1.31×10^{-3}	0.83	0.572
	Habitat $\times$ site	4	1.58×10^{-3}		
	Date	5	6.54×10^{-3}	6.05	0.000
	Date $\times$ habitat	5	2.14×10^{-3}	1.98	0.103
	Habitat $\times$ site $\times$ date	40	8.65×10^{-3}		
Iowa	Habitat	2	1.32	14.68	0.005‡
	Site (replicate)	3	1.47×10^{-1}	1.06	0.433
	Habitat $\times$ site	6	2.79×10^{-1}		
	Date	6	1.39	10.96	0.000
	Date by habitat	12	7.34×10^{-1}	2.58	0.005†
	Habitat $\times$ site $\times$ date	47	1.01		

''Habitat'' main effect refers to different habitat types, and ''site'' refers to locations within a region. ''Date'' is included as a categorical variable. Significant main and interaction effects were compared by using Bonferroni tests.
*Maryland airport site had higher egg densities than Wye Island site.
†Significant date $\times$ habitat interactions in Maryland are caused by higher egg densities in edge sites 21–25 August, in Minnesota/ Wisconsin by higher densities in cornfields 17 July–5 August, and in Iowa by higher densities in corn and bean fields 24 July–4 August.
‡Minnesota/Wisconsin cornfields had higher egg densities than edge or nonagricultural habitats. Iowa corn and bean fields had higher densities than edge habitats.

tural habitats were about 2 and 4 times as productive as cornfields in Minnesota/Wisconsin and Iowa, respectively. When the amount of landscape that includes agricultural and nonagricultural land is taken into consideration, we estimate ≈73 (in Minnesota/ Wisconsin) or 45 (in Iowa) times more monarchs come from cornfields than from nonagricultural habitats. In Ontario, we estimate that cornfields produce ≈18 times fewer monarchs than nonagricultural areas. The overall contribution of field edges cannot be estimated because of a lack of data on their relative areas.

Phenological Overlap Between Monarch Larvae and Pollen Shed. In Minnesota and Ontario, there was substantial overlap (40% and 62%, respectively) between the peak of the migratory monarch generation and pollen shed, whereas pollen shed occurred before the peak of the final generation in Iowa and Maryland (15% and 20% overlap, respectively; Fig. 1 and Table 1 show egg peaks and anthesis dates). Estimates of the proportion of first and second instar larvae in cornfields that are exposed to corn pollen are shown in Table 5.
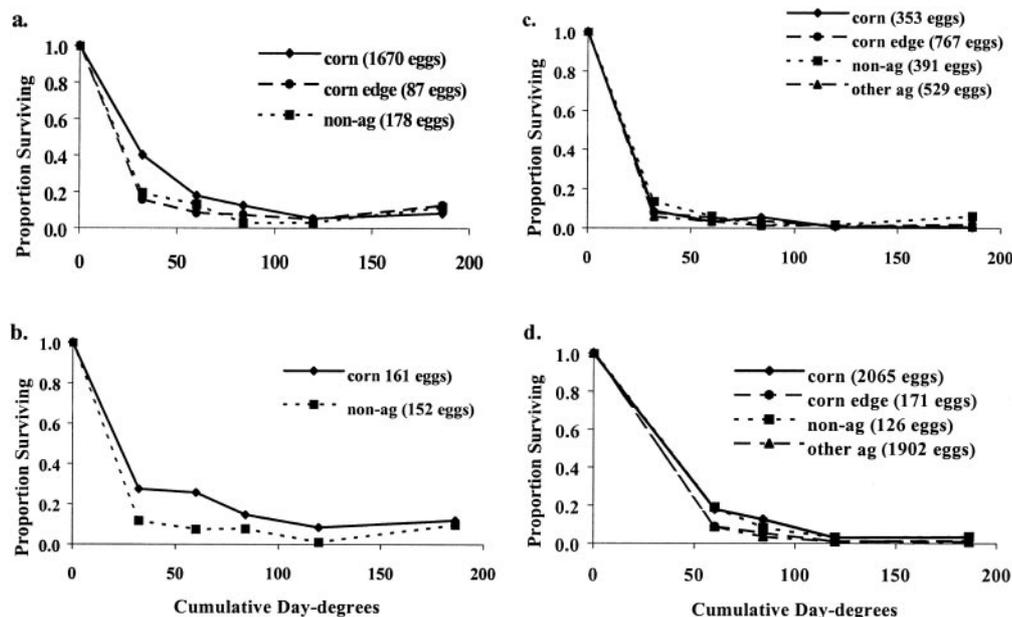

Fig. 3. Survival calculated as the proportion of each stage observed relative to the number of eggs observed in (a) Minnesota/Wisconsin, (b) Ontario, (c) Maryland, and (d) Iowa. Egg numbers are in parentheses. Data on first instars were not used in Iowa calculations because of inconsistencies in identifying this life stage.

Table 3. Weibull parameters comparing survival in different habitats

Region	Habitat type	b	SE	c	SE
Minnesota/	Corn	34.20*	3.725	0.8111	0.1330
Wisconsin	Edge	7.15†	4.331	0.3771	0.1020
	Nonagricultural	10.08†	4.011	0.4340	0.0951
Iowa	Corn	30.55*	9.390	0.7850	0.2550
	Edge	16.68†	3.988	0.6880	0.1120
	Nonagricultural	36.07*	8.018	1.0290	0.3235
	Other agricultural	22.74*†	3.198	0.9187	0.1186
Maryland	Corn	5.78*	2.084	0.4970	0.0861
	Edge	5.29*	1.480	0.4803	0.0613
	Nonagricultural	8.32*	4.458	0.5198	0.1580
	Other agricultural	5.04*	1.750	0.5383	0.0890
Ontario	Corn	19.68*	6.304	0.4045	0.0944
	Nonagricultural	6.00†	3.674	0.4022	0.1059

Welch's unpaired t test with Bonferroni adjustment used for multiple comparisons of model parameters within each region. Values followed by the same superscript are not significantly different at the adjusted confidence level ($P < 0.05$ or less, depending on number of comparisons).

Table 5. Phenological overlap (%) between first and second instars and corn anthesis

	% Overlap			
Site	Minnesota/Wisconsin	Ontario	Maryland	Iowa
1	36	59	12	14
2	68	81	13	19
3	20	27	—*	11
4	44	66	57	21
5	30	75	0	9
Average	40	62	20	15

Estimates for Maryland and Ontario are likely overestimates because monitoring stopped before the end of the final generation.

*No first or second instars were observed in Maryland Gannon site during the entire summer; it was not included in the average for that region.

Discussion

Importance of Agricultural Fields to Monarch Production. We found immature monarchs in cornfields throughout their breeding season, even though the corn is up to 200 cm taller than the milkweed by the end of the season (Fig. 2). In the four regions studied, per ramet densities of monarchs were as high or higher within cornfields as in other habitats on many monitoring dates. Egg densities on milkweed in agricultural fields were even higher relative to nonagricultural habitats at the end of the summer in the upper Midwestern sites (Fig. 1 and Table 2).

Our data suggest a significant proportion of the monarchs that originate in the Midwestern U.S. come from agricultural habitats. We estimate that cornfields and soybean fields together produce 78 times more monarchs than nonagricultural habitats in Iowa, and that cornfields produce 73 times more monarchs than nonagricultural habitats in Minnesota/Wisconsin. Nonagricultural habitats in the upper Midwest tend to produce more monarchs on a per area basis, but they are relatively rare, comprising <1% of the total potential monarch breeding habitat (Table 4), and thus are less important for overall monarch production. In Ontario, the landscape is more heterogeneous, with a greater proportion of nonagricultural habitats. Thus in Ontario, as least for the year in which the study was done, milkweeds in cornfields were not important contributors to monarch production.

Our productivity estimates are based on oviposition, but differences among habitats in the conversion of eggs into adults could affect monarch productivity. Larva survival data (Fig. 3 and Table 4) suggest that monarchs may suffer less early mortality in cornfields, at least in Ontario and Minnesota/Wisconsin. This difference may be a result of lower predator numbers in the less diverse cornfield habitat. However, by the end of the larva period, there were equal proportions of monarchs surviving in all habitats, thus our estimates use relative egg numbers.

These findings demonstrate that practices affecting milkweed densities in agricultural habitats are likely to have large impacts on monarch abundance. Farming practices such as tillage, herbicide use, and cropping choices may affect milkweed abundance and thus monarch numbers. Effects of tillage practices are unclear; Yenish *et al.* (20) found that fields in which conventional tillage is practiced have lower milkweed densities than fields undergoing conservation tillage practices, whereas Swanton *et al.* (21) found no consistent effect of tillage system on weed density, including milkweed, in corn. A possible reason for the low

Table 4. Estimated milkweed density and monarch production over entire period monitored

Region/habitat	No. milkweed observed	Eggs/ milkweed	Milkweed density in study sites	Landscape milkweed density*	Relative monarch productivity†	Proportion of breeding habitat‡	Relative contribution of habitat§
Maryland							
Corn	25,566	0.0137	0.004/m²			<0.001	
Other agricultural	13,980	0.0378	0.003/m²			0.988	
Edge	13,425	0.0571	0.039/m²				
Nonagricultural	10,847	0.0360	0.027/m²			0.0123	
Minnesota/Wisconsin							
Corn	27,388	0.0612	0.285/m²	30 m²/hectare	0.65	0.124	73
Edge	5,493	0.0149	0.525/m²	102 m²/hectare	0.54		
Nonagricultural	13,257	0.0134	1.052/m²	212 m²/hectare	1	0.0011	1
Ontario							
Corn	12,125	0.0125	0.272/m²	0.008/m²	0.0081	0.302	0.055
Nonagricultural	12,097	0.0133	3.604/m²	0.924/m²	1	0.045	1
Iowa							
Corn	10,846	0.190		30 m²/hectare	0.24	0.436	45
Beans	9,124	0.208		16 m²/hectare	0.14	0.562	34
Edge	10,452	0.0313		102 m²/hectare	0.13		
Nonagricultural	4,337	0.114		212 m²/hectare	1	0.0023	1

*Ontario estimates from random field survey in Ontario, and Iowa and Minnesota/Wisconsin from Hartzler and Buhler (10). Note that units are different in ref. 10; single stems or clumps of milkweed were assigned patch sizes of 1 m².

†Productivity on a per area basis. Values obtained by multiplying columns 3 and 5, then relativizing to nonagricultural habitat.

‡U.S. values from Taylor and Shields (15). Ontario values from refs. 16 and 17.

§Relative contribution on a landscape basis calculated from egg densities and landscape data (contribution = relative productivity × proportion of breeding habitat), relativized to nonagricultural land.

densities of milkweed in Ontario and Maryland cornfields may be reduced tillage, which could favor clumped milkweed growth, thus making it easier to control (C. Swanton, personal communication). Additionally, more diverse cropping systems (as opposed to the corn–soybean rotation common in the upper midwestern U.S.) or herbicide use may lower milkweed densities in Maryland and Ontario cornfields.

Our conclusions require some caveats. More accurate estimates of the relative importance of different habitats will require extensive and random surveys of milkweed densities on a landscape scale, using consistent sampling methods; the methods used in Ontario for this study and by Hartzler and Buhler (10) were different, and these studies covered only a small portion of the monarch's breeding range. Our cornfields tended to have higher milkweed densities than random fields (Table 4), and this may have affected female oviposition behavior; however, the lack of a correlation between milkweed and monarch density suggests that nonrandom site selection may not have affected our results. Better information on land-use patterns will also add to the accuracy of estimates of relative productivity. Our observations were made during a single growing season; repeating the observations would allow us to generalize our results. Finally, calculations of relative productivity are reported without associated error terms and thus cannot be compared statistically. Fig. 1 illustrates the error associated with egg densities in each region; standard errors of milkweed densities in the field surveys in Ontario and Minnesota/Wisconsin ranged from 25 to 45% of the means. The Hartzler and Buhler (10) data on milkweed densities used for the upper Midwest were reported without error (15). Our goal was to estimate the relative importance of different habitat types, and our estimates suggest differences within and between regions ranging from one to two orders of magnitude (Table 4). This is the important finding, and more exact estimates will require additional data.

Despite these caveats, our study clearly indicates an assessment of risks imposed by Bt corn must consider pollen densities that fall on milkweed within cornfields. The small area of cornfield edge habitat, relative to the area of the fields themselves, and lower amounts of pollen that fall onto host plants outside the fields, make field margins less important in risk assessments.

Probability of Exposure to Bt Corn Pollen. In the northern study sites (Ontario and Minnesota/Wisconsin), the peak of the migratory monarch generation coincided with pollen shed in 2000, leading to the large phenological overlap shown in Table 5. This overlap was lower in Iowa and Maryland, where anthesis occurred before the peak of the migratory generation. The exposure this represents on a population-wide basis will depend on the proportion of larvae in cornfields and the proportion of fields planted in Bt corn. We can use Iowa, the region for which we have the most complete data, as

an illustrative example. If we assume that all monarchs from Iowa come from cornfields, soybean fields, or nonagricultural areas, the relative proportions of monarchs in the final column of Table 4 suggest that 56% of them originate in cornfields (45 is 56% of the total relative productivity of 45 + 34 + 1). If 15% of these are exposed to corn pollen (Table 5), and 35% of that pollen is from Bt corn (based on industry sources for the proportion of fields in Iowa planted to Bt varieties), then ≈3% of the monarchs emerging in Iowa over the course of a summer could be exposed to Bt pollen. This percentage will be higher further north, such as in Minnesota and Wisconsin where phenological overlap is ≈3 times greater (Table 5). Including later stadia in calculations would also increase the estimated overlap, but we did not do this to avoid counting individuals more than once.

Our measurement of corn anthesis occurred only at weekly intervals, thus the window of anthesis we measured in each field may be substantially longer or shorter than if our observation intervals had allowed for daily resolution. The time frame is consequential, because monarch life stages change on a scale of days rather than weeks. In addition, an accurate exposure assessment will require longer term data, because year to year temperature variations will affect corn and monarch phenology.

Summary

Results presented here have two important implications. First, a portion of the monarch population is exposed to and probably consumes corn pollen that collects on milkweed plants growing in cornfields. Recent research suggests that the Bt corn hybrids most commonly planted produce levels of toxin in their pollen that are unlikely to have severe fitness consequences on monarchs (22–24), but our findings indicate the need to evaluate future transgenic hybrids on the basis of their protein toxicity and expression in pollen. Second, regardless of risks imposed by transgenic corn, changes in agricultural practices such as weed control or the use of foliar insecticides could have large impacts on monarchs by affecting milkweed density and condition, or monarch survival.

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