Preliminary Report on the Ecological Impact of BT Corn Pollen on the Monarch Butterfly in Ontario

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Executive Summary

Objectives

- To determine a dose-toxicity relationship for Bt 176 corn pollen and monarch larvae.
- To determine the spatial arrangement of milkweed in relation to corn fields.
- To investigate the extent of dispersal of corn pollen from corn fields and the concentration of corn pollen that actually adheres to milkweed foliage at different distances from fields.
- To examine the abundance and phenology of monarchs developing on host plants within the range of significant levels of pollen drift from Bt corn.

Toxicity of Bt corn pollen to monarch larvae

- The LD_{50} (the lethal dose required to kill 50% of the larvae) was 389 Bt 176 pollen grains/cm^2 at 96 hours, following a 48 hour exposure period. No decreases in larval survival were observed following exposure to 133 Bt 176 pollen grains/cm^2 leaf material and no decreases in survival were observed following exposure to non-Bt pollen at any of the doses studied.
- Our data do not provide conclusive evidence for a delay in development following ingestion of Bt or Non-Bt pollen compared with larvae fed only leaves.
- Overall, exposure to low doses of Bt pollen resulted in decreased weight gain by day 5 when compared with weight gain of larvae fed leaves only, while low doses of non-Bt pollen did not result in lower weight gain.
- Although no decrease in consumption was observed after 48 or 96 hours for larvae fed non-Bt pollen, larvae fed Bt-pollen ate less leaf material than those fed only leaves.
- Our data do not provide evidence for an avoidance of Bt by 1st instar larvae through physical movement away from the pollen-dusted surface.
- The results of the bioassay are extremely preliminary and should be interpreted with caution.

Exposure of monarch larvae to Bt corn pollen

- The vast majority of pollen fell within a few meters of the corn field (approximately 90% falls within 5 meters).
- On average, pollen counts on leaves were lower than those demonstrated to be toxic to neonates less than one week following peak pollen shed (although the range of values included densities that approached the LD_{50} determined in our experiments). Further, only 5 meters from the field, pollen counts on milkweed leaves were close to zero.
- More milkweed plants occur in conservation areas compared to cultivated areas excluding roadsides. The importance of milkweed plants along roadsides requires further study.
- Preliminary data do not provide evidence for a strong phenological overlap between monarch larval stages and peak pollen shed in Ontario, 1999.

Section I: Background

I. Introduction:

In response to a recent publication in the journal *Nature*[1] that demonstrated detrimental affects in larvae of the monarch butterfly (*Danaus plexippus*) following ingestion of Bt corn pollen, a CFIA-supported research project was initiated at the University of Guelph to further investigate the impact of Bt corn pollen on non-target Lepidoptera. The study published in *Nature* found that exposure to pollen from transgenic corn plants expressing a *Bacillus thuringiensis* endotoxin from a Bt-11 hybrid (N4640-Bt corn) resulted in increased mortality and delayed development compared with ingestion of non-Bt pollen. Decreased consumption was observed following ingestion of either pollen type compared with control larvae that were fed only leaves. Because the amounts of pollen used in the aforementioned study were not reported, and because the level of exposure of non-target lepidopteran species to toxic doses of Bt pollen is unknown, the relevance of the toxicity of Bt-corn pollen to monarch larvae has yet to be determined. Thus, the current investigation aims to further refine the dose-toxicity relationship and to predict the level of exposure of monarch larvae to Bt corn pollen in Southern Ontario. The risks to non-target lepidoptera depend on numerous factors, some of which are addressed here, others in the future.

Some factors affecting toxicity:

- differential sensitivities of various larval instars
- the age of the pollen (to account for degradation of the endotoxin)
- the source of pollen (four of five Bt-corn events will be represented in the final report)

Some factors affecting exposure:

- the overlap between the presence of sensitive larval instars and the pollen shed period for corn in Ontario
- the proportion of suitable habitat in close proximity to corn
- the distance that pollen disperses from the field
- the degree to which pollen collects and persists on milkweed
- dispersal and degradation patterns under different weather conditions
- antixenosis and oviposition deterrence.

[1] Losey, J. E., Rayor, L. S. and M. E. Carter. 1999. Transgenic pollen harms monarch larvae. Nature, 399, p. 214.

II. Objectives addressed between July 1999 and December 1999:

- To determine a preliminary dose-toxicity relationship for Bt corn pollen and monarch larvae using field collected pollen and larvae in laboratory bioassays
- To determine the spatial arrangement of milkweed in relation to corn fields.
- To investigate the extent of dispersal of corn pollen from corn fields and the concentration of corn pollen that actually adheres to milkweed foliage at different distances from fields.
- To examine the abundance and phenology of monarch butterflies developing on milkweed plants within the range of pollen drift from Bt corn grown under typical field conditions.

Section II. Toxicity of Bt Corn Pollen to Monarch Larvae

I. Introduction:

Preliminary bioassays were conducted using monarch larvae. In order to assess the "worst-case scenario" for toxicity of Bt corn pollen to the larvae, we used 1st instars and exposed them to Bt-176 pollen (Maximizer357, Novartis Seeds). Neonates are often more sensitive to toxic compounds than are larger larvae and Bt-176 has greater concentrations of Bt endotoxin than either Bt-11 or Mon810[1].

II. Methods for Collecting Pollen and Conducting Bioassays:

Pollen Collection

During the peak pollen shed period (a five day period between July 15 and July 22, 1999 depending on the field location), pollen was collected by stapling paper bags over the shedding tassels of 100 randomly selected corn plants at each of the nine field sites. After 24 hours, the bag and tassels were removed from the plant and taken back to the laboratory where tassels were shaken out and removed and the pollen and anthers were collected in clear plastic containers with ventilated lids. Pollen collected from each of 25 plants was pooled in a single container so that there were four containers per field. The pooled

[1] The Canadian Decision Document (Health Canada) reports that Bt-176 corn pollen (Novartis Seeds) contains 1.4 to 2.3 µg Bt toxin/g pollen Bt-11 and Mon810 events are reported to contain 0.33µg toxin/g pollen and 0.09µg toxin/g pollen respectively (Laura Privalle, Novartis Seeds, Pers. Comm. , Mark Groth, Monsanto Co., Pers. Comm.)

pollen samples were then aged for 0, 2, 5 or 10 days prior to being stored in a freezer at -17°C. Pollen was aged by placing the containers outside on a green background in a sunny location. Containers were covered with a tarp overnight and during periods of rainfall. After 2, 5 or 10 days, containers were sealed and placed in the freezer.

Bioassay Methodology

Bioassays were conducted in a growth chamber under uniform conditions. Larvae were exposed to pollen in arenas that consisted of ventilated Petri dishes (13.5cm diameter), each containing a milkweed leaf to which a known density of pollen had been applied using a modified Potter tower. The tower was modified by replacing the nozzle through which a liquid insecticide is normally administered with a mesh basket on which a known amount of pollen was placed. Pollen was forced through the mesh by allowing a stream of air to flow through a glass funnel cupped over the basket. This method resulted in an even distribution of pollen grains over a leaf placed at the bottom of the tower. Ten larvae ≤ 24 hours old were weighed as a cohort and placed in the centre of the top of a leaf on which either Bt pollen (Bt 176 from Max357 hybrids, Novartis Seeds, Inc.), non-Bt pollen (EnerFeast 1, Novartis Seeds Inc.) or no pollen (control) was applied. Larvae were allowed to feed on the treated leaflets for 48 hours, after which they were given fresh, untreated leaf material daily. Leaf consumption was measured after 48 hours (for the entire exposure period) at 96 hours (encompassing the previous 24 hours) and after 7 days (encompassing the previous 24 hours) using a digital camera attached to a computer containing area analysis software. Weights of larvae were recorded for each cohort on the fifth day. Development, mortality and the position of the larvae on the leaves (top or bottom) were recorded daily. The initial measure of consumption allowed us to estimate the amount of pollen (and therefore the amount of toxin) consumed by each cohort.

Estimated intake of toxin by neonate larvae following a 48 hour exposure period to Bt-176 pollen.

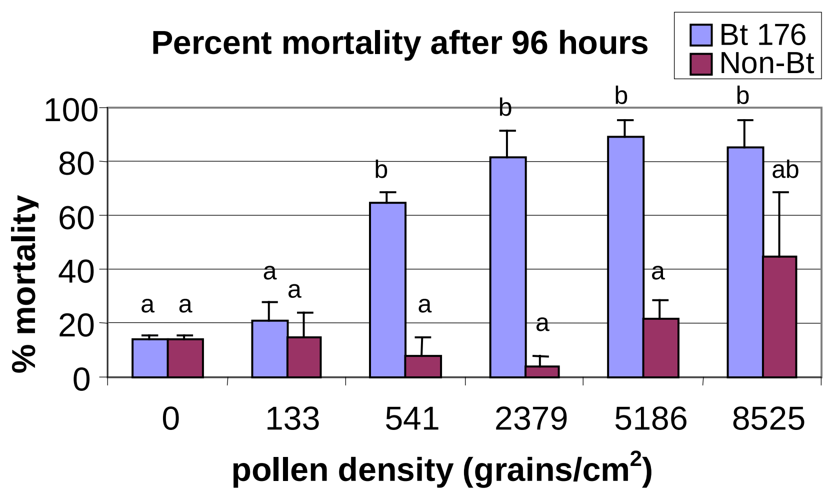
Pollen Density (grains/cm^2)	Dose of Bt-176 (µg endotoxin/larvae)[1] Mean±SE
0	0
133	7.1E-06 ± 1.9E-06
541	1.3E-05 ± 8.1E-06
2379	1.8E-05 ± 1.1E-05
5186	6.4E-05 ± 3.4E-05
8525	2.5E-04 ± 1.8E-04

[1] Based on weight of pollen eaten (the leaf area consumed per larvae x the pollen density x the weight for a single pollen grain (based on a calculated weight/density relationship)) and the concentration of endotoxin in the pollen.

III. Effects of Bt 176 Pollen on Survival of 1st instar monarch larvae:

At 133 Bt-176 pollen grains/cm^2, no decreases in larval survival were observed in preliminary bioassays

Analysis by Polo-PC software revealed that the LD_{50} (the lethal dose required to kill 50% of the larvae) was 389 grains/cm^2 at 96 hours, following a 48 hour exposure period. An ANOVA (SAS, 1991) revealed an increase in mortality following doses of Bt corresponding to 541 grains/cm^2 or greater but not 133 grains/cm^2 when compared with larvae fed leaves only. No differences in mortality were observed for larvae fed non-Bt pollen compared with those fed leaves without pollen.



Data were analysed using an ANOVA followed by Tukey's Studentized Range (HSD) multiple comparisons test (SAS, 1990)[1].

IV. Sublethal effects of Bt 176 Pollen on 1st instar monarch larvae:

Preliminary bioassays do not provide evidence for a delay in development time following ingestion of Bt-176 pollen

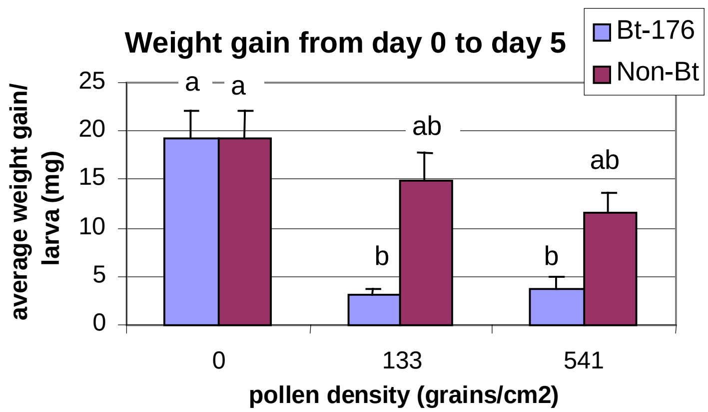
Because many of the doses used in the bioassays caused complete or near mortality of larval cohorts, analysis of sublethal effects was limited to doses from 0 to 541 pollen grains/cm^2. For this reason, it

[1] ... Guide fourth edition. SAS Institute Inc., Cary NC, USA.

is not a complete test of the influence of Bt corn pollen on sublethal effects. Our data do not provide evidence for a delay in larval development following ingestion of Bt or Non-Bt pollen compared with larvae fed only leaves. The proportion of larvae that reached at least the second instar by day 4 or that reached the fifth instar by day seven did not differ significantly across treatment groups.

Low doses of Bt-pollen caused decreased weight gain.

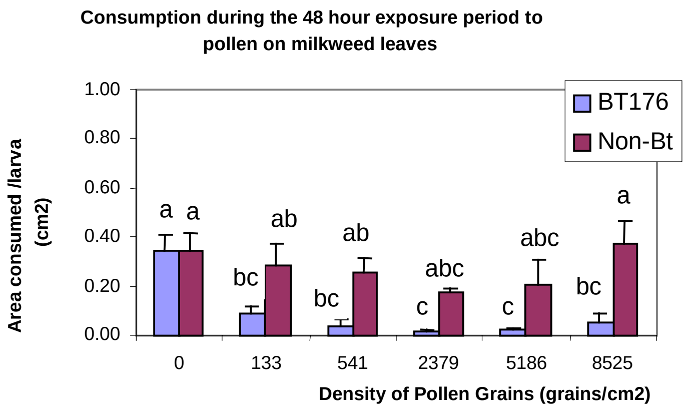
Exposure to low doses of Bt pollen resulted in decreased weight gain by day 5 while no effects of non-Bt pollen were observed at these doses. High doses of Bt pollen resulted in mortality levels that precluded analysis.

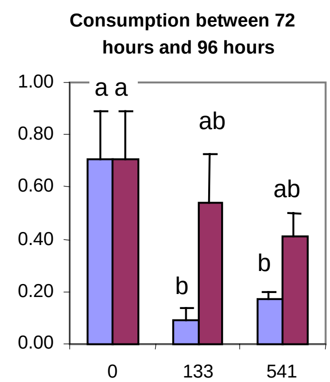


Data were analyzed using an ANOVA followed by Tukey's Studentized Range (HSD) multiple comparisons test. Significant differences ($p \leq 0.05$) amongst larvae fed the low doses of pollen tested are indicated. There were only enough survivors in cohorts in the 3 doses presented above to be included in the analysis.

Bt-176 pollen caused decreased consumption at all doses

Although no decrease in consumption was observed for larvae fed Non-Bt pollen at either 48 or 96 hours, larvae fed Bt-pollen ate less leaf material than those fed only leaves at all pollen doses. Again, high doses of Bt pollen resulted in high mortality levels by day 4, precluding analysis for consumption at 96 hours.





Data were analysed using an ANOVA followed by Tukey's Studentized Range (HSD) multiple comparisons test (SAS, 1991). Consumption periods were analysed separately and data for the three highest doses in the latter period were not included in the analysis due to high mortality levels at 96 hours. After 96 hours, there were only enough survivors in the cohorts presented above for consumption between 72 and 96 hours for analysis.

Avoidance of Bt-176 pollen was not observed

After 48 hours there were no significant differences in the number of larvae found on the upper leaf surface (which contained pollen) compared with other locations (the lower leaf surface or elsewhere in the dish) for larvae exposed to Bt-176 pollen, non-Bt pollen or no pollen ($p>0.05$). Thus, our experiment provides no evidence for an avoidance of Bt by 1st instar larvae through physical movement away from the pollen-dusted surface.

V. Conclusions:

- Pollen from a Bt-176 hybrid was found to be toxic to neonate monarch larvae at most doses studied. Given that the Bt endotoxin in transgenic corn was selected on the basis of its toxicity to Lepidopteran insects (particularly, the European corn borer, *Ostrinia nubilalis*) these results are not surprising.

- At doses that did not cause an increase in mortality, sublethal effects were observed including decreased consumption and weight gain compared with control larvae and larvae fed non-Bt pollen. Our data do not support a significant decrease in developmental time for larvae fed Bt pollen versus non-Bt or no pollen. Our results are similar to those of Losey et al. (1999)[1] except for the finding by Losey that larvae fed non-Bt pollen also consumed less than those that were not fed pollen. Differences in the types of pollen used and experimental methodology may explain this discrepancy.

- The results of the bioassay are preliminary and as such, should be interpreted with caution. Our research is ongoing and further data will improve our assessment of lethal doses and sublethal effects of Bt pollen on the monarch butterfly.

VI. Future research Goals:

Beginning in February, bioassays will be conducted that include a range of sublethal doses in addition to lethal doses in order to establish a more refined threshold dose for mortality and in order to assess potential sublethal effects on larvae and adult butterflies exposed to Bt pollen as larvae. The relevance of sublethal effects to the population will depend

[1] Losey, J. E., Rayor, L. S. and M. E. Carter. 1999. Transgenic pollen harms monarch larvae. Nature, 399, p. 214.

on both the reproductive fitness of exposed insects and the possible effect on reproductive and/or migratory synchrony with non-exposed individuals. Bioassays will also be conducted using third instar monarch larvae and pollen from other events.

In addition to continued laboratory bioassays, a field bioassay will be conducted during the summer, 2000 in which first and third instar monarch larvae will be placed on milkweed plants at known distances from corn fields and mortality and development monitored to pupation. Surviving fifth instar larvae will be brought to the lab to complete development so that the fecundity of adults can be assessed. A field evaluation is imperative in order to account for natural and behavioural factors that cannot be simulated in a laboratory experiment.

Section III. Exposure of Monarch Larvae to Bt Corn Pollen

I. Introduction:

The severity of detrimental effects of Bt corn pollen on non-target Lepidopteran species depends on the level of exposure to toxic doses of pollen. Thus, in the summer of 1999, we examined the distance, direction and density of pollen dispersal at several field sites in Southwestern Ontario. In addition, we collected milkweed leaves at known distances from the field edge and determined pollen deposition on them at the end of the pollen shed period. In order to determine the extent to which milkweed occurs in cultivated and uncultivated areas, transect counts of plants were taken in various habitats adjacent to corn fields. Finally, separate transects in three habitats were used to investigate the phenological overlap between monarch larvae and pollen shed.

II. 1999 Field Locations:

Nine Bt-corn fields were chosen for study which were located within the Wellington, Oxford and Hamilton-Wentworth counties of Southern Ontario. An attempt was made to select small corn fields (< 50 acres) with access to all sides and little to no adjacent corn. When this was not possible, fields were selected that met these criteria on the southern and eastern sides, as these were considered the most important for study due to prevailing Northwesterly winds. The study was conducted during the pollen shed period from July 16 to July 31, although specific timing of the shed varied from field to field.

The fields were primarily rectangular and, ideally, were to have 16 transects radiating from the field's center and passing through either the four corners, or ¼, ½ or ¾ of the way down each side. Along each transect, a wooden stake (approximately 1 m high) was placed at 0, 1, 5, 10, 25, 50 and 100 m from the field's edge. Adjustments were made to these distances along angled transects in order to maintain the correct distance perpendicular to the edge of the field. In many cases, the property around the study field was inaccessible (i.e., residential, dense forest, harvesting field crop) and only the stakes closest to the edge could be erected.

Because pollen shed occurs over approximately 2 weeks, with a peak period of shed during the first 5 days, pollen dispersal was sampled over 24 hour periods for the first five days and then over 48 hour periods for the remainder of the shed. Pollen was collected on Petri plates painted with sticky material (Sticky Stuff or Tanglefoot) that were attached to the top of each stake with velcro. After exposure, plates were labeled, replaced with a new plate and held at –17° C to prevent mould growth. Pollen was counted on each plate at 5 randomly selected $1 cm^2$ areas and a mean pollen density per square centimeter was determined.

III. Distance and Direction of Pollen Dispersal:

Pollen counts on Petri plates are currently underway. Thus far, data for the first three days for each of two fields has been recorded and is presented below.

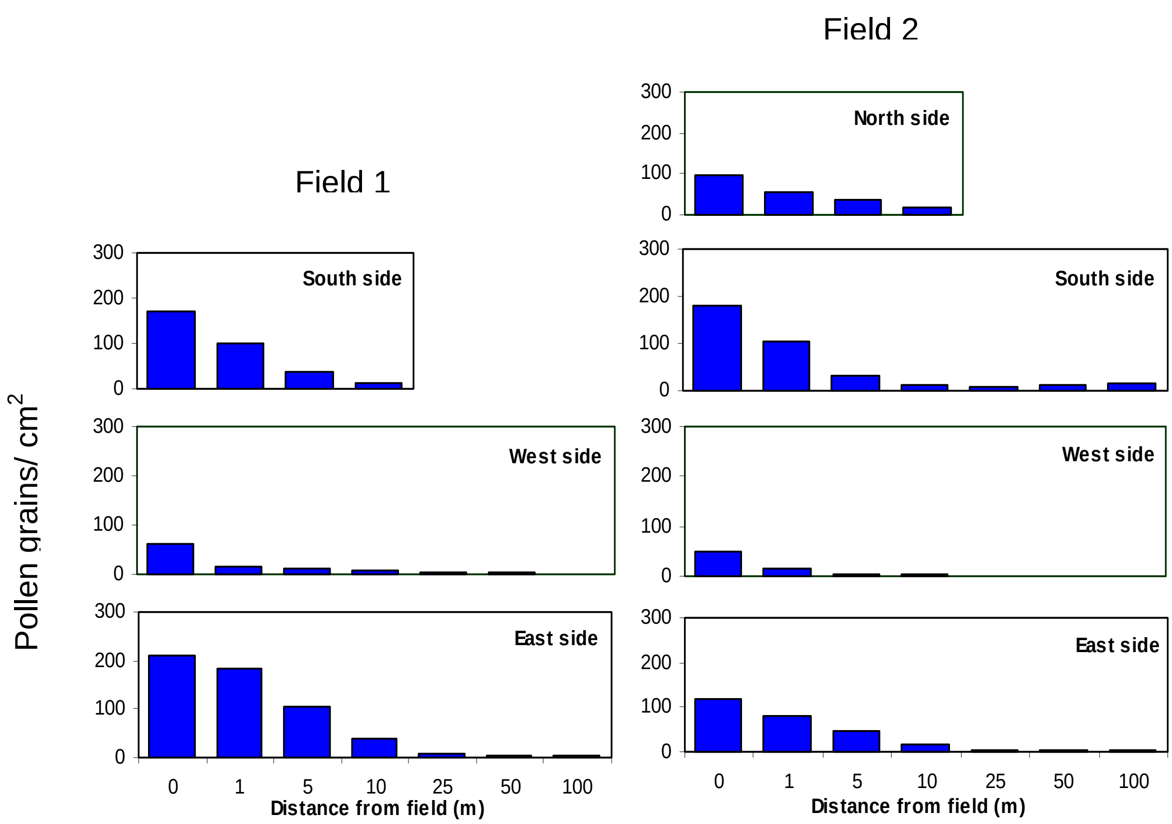
Field	Direction	Percent pollen falling within a given distance from the field edge*	
		1 m	5 m
1	S	84	96
	W	75	87
	E	71	90
2	N	74	92
	S	78	87
	W	82	87
	E	72	89

* data are averages of pollen counts for the plates collected from July 16 to July 18. For each plate, 5 randomly selected $1 cm^2$ areas were observed.

Regardless, of the direction from the field, most of the pollen falls within 5m of the field's edge. This data is remarkably consistent with the findings of numerous other researchers in the United States [1].

The direction of pollen dispersal from the field edge reflects the prevailing Northwesterly winds in Southern Ontario. Thus, the south and east sides of a field tend to collect more pollen outside of the field than the north and west sides.

[1] Research findings presented at the Monarch Butterfly Research Symposium, Chicago, 1999 by Dr. Richard Hellmich, USDA, Iowa State University; Dr. Galen Dively, University of Maryland; and Dr. John Pleasants, Iowa State University were remarkably consistent with our own findings.



Pollen dispersal over the first three days of peak pollen shed (July 16-18) for two fields in the summer of 1999

For the east side of Field 1 (which collected more than 5x the amount of pollen found on the west side), the actual amounts of pollen found on plates over a 24 hour period at the edge of the field for each of three days of the peak pollen shed period ranged from 158 to 266 grains/cm^2, while the amounts recorded from plates positioned 5m from the east edge ranged from 34 to 175 grains/cm^2. On average, 209 grains/cm^2 and 104 grains/cm^2 were found at the edge and at 5m respectively. At 10 m from the field edge, an average of 38 grains/cm^2 was observed for this field.

These values are important for our understanding of the relevance of doses found to be toxic to monarch larvae. However, they must be balanced against factors such as pollen accumulation over several days and rainfall or wind patterns that may decrease the densities found on leaves. Preliminary results from the work of Pleasants et al. (1999)[1], indicate that milkweed leaves accumulated only 30% of that

[1] J. M. Pleasants, R. L. Hellmich., and L. C. Lewis. Pollen deposition on milkweed leaves under natural conditions. Presentation at the Monarch Butterfly Research Symposuim, Chicago, Nov. 1999.

collected from sticky slides and that a heavy rainfall reduced the amount of pollen on leaves by about 90%.

IV. Pollen Accumulation on Milkweed Leaves from Plants within 5m of Corn Field Borders:

Pollen density on milkweed leaves was determined at three distances from the field's edge. Leaf samples were taken between July 26th and July 28th from four fields for which peak pollen shed (first 5 days of pollen shed) ended on either July 20th or July 21st. Ten leaves were randomly sampled from milkweed plants inside the first corn row and from plants at 0 and 5 m from the field edge (n = 120 leaves). Each leaf was placed on cardboard, covered with plastic wrap, pressed and chilled until analysis. Pollen was counted in 10 randomly selected $1cm^2$ areas. Although no direct comparisons can be made between counts on Petri plates versus leaves, the leaf counts provided an indication of the persistence of pollen on leaves following peak pollen shed but prior to the end of the pollen shed period. A heavy rainfall occurred on July 19th and July 20th, thus, the amounts on leaves essentially represent 6 to 8 days of pollen accumulation.

Pollen Density on Milkweed Leaves Collected from the Southeast side of four Bt corn fields in Ontario

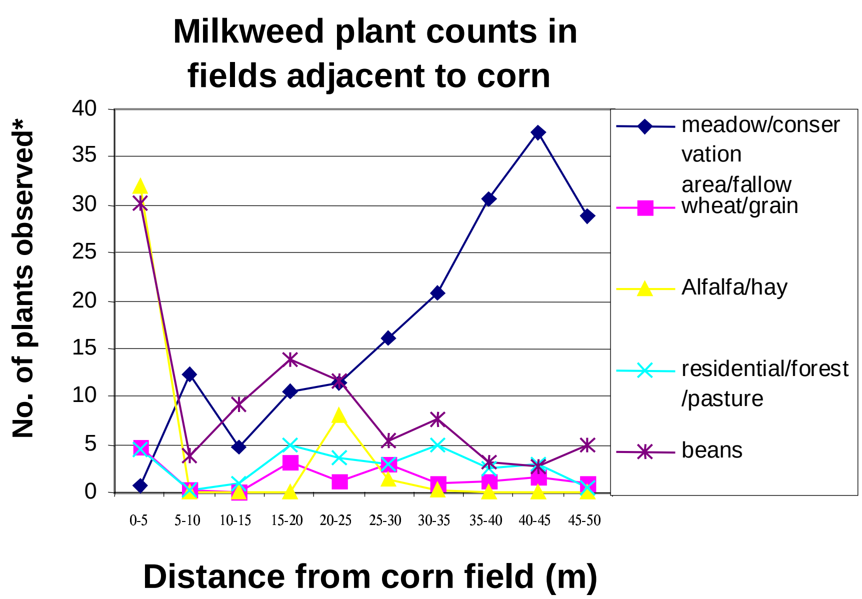
Distance from Border of Corn Field (m)	Pollen Grains per cm^2 Mean ±SE	Range
-1 to 0	78 ± 9.5	1 - 381
0 to +1	28 ± 7.3	0 - 356
5	1.4 ± 1.8	0 - 6

Though preliminary, these results indicate that 1 meter from the field, leaves were highly unlikely to contain toxic doses of pollen and that no leaves sampled from plants at 5 meters from the field contained toxic doses of pollen. These data suggest that the risks to monarch larvae may be low, although a closer examination of pollen deposition on leaves during peak pollen shed, an assessment of the importance of sublethal effects of Bt pollen and an assessment of the importance of milkweed plants in field margins are necessary.

The results are consistent with those of other researchers. For example, Dr. Galen Dively (University of Maryland) found that within 10 feet from the field edge inside the field, the average pollen count on leaves was 43 grains/cm^2. Within 10 feet outside of the field, levels were 17 grains/ cm^2. Similar results were also observed by Dr. John Pleasants (University of Iowa) and Dr. Richard Hellmich (USDA, Iowa State University)[1].

V. Proximity of milkweed to Corn Fields in Ontario:

In order to determine the numbers of milkweed plants in various habitats around corn fields, we recorded the numbers of plants along transect lines for 50 fields in Southwestern Ontario. Milkweed plants were counted along four transects perpendicular to corn field edges at randomly selected points parallel to the field edge. A two meter measuring stick was carried along the transect and plants falling within 1 meter on either side of the transect line were included in the counts up to a distance of 50 meters. Thus, the total area covered per field was 400m^2. Ten fields were sampled for each of five habitats. The numbers of milkweed plants found in five different habitats are depicted below.



* Numbers are averages for 10 fields and represent plant counts at four 2m wide transects for each 5m interval.

1 J. M. Pleasants, R. L. Hellmich., and L. C. Lewis. Pollen deposition on milkweed leaves under natural conditions. Presentation at the Monarch Butterfly Research Symposuim, Chicago, Nov. 1999. Dively, G. Deposition of corn pollen on milkweeds and exposure risk to monarch bbutterfly larve in Maryland. Presentation at the Monarch Butterfly Research Synposium, Chicago, Nov. 1999.

The data indicate that meadows/conservation areas contain higher numbers of milkweed plants than other habitats as one moves away from the field edge. Not shown above, however, are the numbers observed on either side of roadways. For our data, the average number of plants observed within ten meters of roadways adjacent to corn fields was 14.3±6.3. (This value is the average number of plants observed for 17 roadways adjacent to corn fields and represents the sum of four 2m wide transects for each five meter interval.) Because plants within fields harvested during the summer cannot be included as suitable habitat, those plants directly adjacent to corn fields and along roadsides may be important in supporting non-target Lepidopteran species. Further investigation is necessary to determine the relative importance of roadside host plants and field margins (subject to heavy pollen deposition) and conservation areas (which contain greater numbers of plants and are less likely to collect pollen) on the populations of non-target lepidopteran species.

Southwestern Ontario represents only 3% of the total land area of Ontario but most of the corn is grown in this region; while corn represents only 0.7% of the land use in Ontario, it represents 18% of the land use of Southwestern Ontario[1]. The area covered by major crops in this region is listed below:

Acreage of Major Crops in Southwestern Ontario.

Crop	Mill. Acres in SW Ontario	% of area in SW Ontario
Total area	7.92	100
Grain corn	1.44	18
Soybeans	1.81	23
Wheat and Grains	1.40	18
Alfalfa and Hay	1.00	12.6
Total Farmland	5.90	74

Much of the land area in Southwestern Ontario is cultivated, thus, the milkweed plants falling within these habitats may comprise a significant proportion of the total number of milkweed plants.

VI. Phenology of Monarchs during and following Corn Pollen Shed

Milkweed plants found along transects that extended 100 meters out from the edge of one of four corn fields were carefully examined for the presence of monarch eggs and larvae. Three types of habitat (soybeans, wheat and clover) were represented and sampling took place

[1] OMAFRA, 1998. Grain Corn – Area and Production, Ontario by County, 1998. Agricultural statistics for Ontario. Http://www. gov.on.ca/OMAFRA/English/stats/crops/

after the peak pollen shed period between July 24th and July 26th. Although numerous data were recorded (including the size and health of plants, the presence of other insects and the distance and direction from the corn field) only the presence and stage of monarch larvae and eggs are presented. Monarch butterflies were observed during and following the pollen shed period but no attempt was made to quantify the numbers of butterflies observed.

Host Plant	Total no. of plants sampled	No. of eggs observed	No. of larvae observed
Milkweed	371	24	2

The current data do not provide evidence for a strong phenological overlap between larval stages and peak pollen shed at the field sites studied. According to the Ontario Butterfly Atlas[1], monarchs tend to occur in the late larval and pupal stages at this time of year. However, we observed adults at our field sites throughout July and eggs in late July indicating that year-to-year fluctuations and differences across the province likely occur. A more detailed phenological study incorporating different heat units across Southwestern Ontario is necessary.

VII. Conclusions:

- The majority of the pollen fell within a few meters of the corn field (approximately 90% falls within 5 meters).

- On average, pollen counts on leaves were lower than those known to be toxic to neonates less than one week following peak pollen shed (though the range of values included densities that approached the LD_{50} determined in our experiments). Further, only 5 meters from the field, pollen counts on milkweed leaves were close to zero.

- More milkweed plants occur in conservation areas compared with cultivated areas excluding roadsides. The importance of milkweed plants along roadsides and field margins requires further study.

- Preliminary data do not provide evidence for a strong phenological overlap between monarch larval stages and peak pollen shed in Ontario, 1999.

[1] Holmes, A. M., Tasker, R. R., Hess, Q. F. and A. J. Hanks. 1991. The Ontario Butterfly Atlas. Toronto Entomologists' Association, Toronto Ontario.

VIII. Future research Goals:

Between January and April 2000, pollen count data collection and analysis will be completed for the nine field sites sampled in the summer of 1999. From this data we will be able to statistically evaluate the dispersal pattern of corn pollen from fields in Southwestern Ontario and correlate these patterns with weather data where possible. During the summer of 2000, a more detailed assessment of pollen dispersal will be conducted at fewer sites that will include a direct comparison between pollen deposition on milkweed leaves compared with that on sticky traps. A more detailed assessment of the populations of milkweed plants in suitable habitats will also be made to determine the spatial arrangement and likelihood of exposure of milkweed plants to pollen. Finally, we will conduct a comprehensive phenological study to determine the overlap between the presence of larval instars of monarchs and the pollen shed period in Ontario.