Management of insecticide resistance in Oriental fruit moth (Grapholita molesta; Lepidoptera: Tortricidae) populations from Ontario

Lambert HB Kanga,1* David J Pree,2 Jennifer L van Lier2 and Gerald M Walker3

1United States Department of Agriculture, Agricultural Research Service, Beneficial Insects Research Unit, 2413 E Highway 83, Weslaco, TX 78596, USA
2Agriculture & Agri-Food Canada, Southern Crop Protection & Food Research Centre, 4902 Victoria Avenue North, PO Box 6000, Vineland Station, Ontario LOR 2EO, Canada
3OMAFRA, Vineland Station, Ontario LOR 2EO, Canada

Abstract: The development of resistance in the Oriental fruit moth, Grapholita molesta (Busck) to organophosphorus (OP) insecticides (azinphos-methyl and phosmet) is a serious threat to the tender fruit industry in Ontario (50% crop losses in 1994). Resistance to carbamate insecticides and increased survival of field-collected moths at diagnostic concentrations of pyrethroids were widespread. As a result, four different treatment regimes were tested to manage resistance in G. molesta, and the changes in resistance frequencies under each treatment regime were monitored from 1996 to 1999. The data indicated that the levels of resistance were significantly influenced by the various treatment regimes. The seasonal pattern of resistance was similar for all treatment regimes, in that resistance peaked in mid-season and declined in the late season. Levels of resistance in G. molesta to OPs decreased from 55% to 14% and that to pyrethroids declined from 30% to 10% from 1996 to 1999 under a treatment regime consisting of endosulfan–organophosphate–pyrethroid rotation. Similarly, under a treatment regime implemented in commercial orchards (organophosphate–pyrethroid rotation), resistance to OP insecticides declined from 50% to 12% and resistance to pyrethroids evolved to around 16%. The overall data indicated that resistance was unstable; a strategy based on rotation of insecticides by class for each generation of G. molesta was successful in managing resistance to both OP and pyrethroid insecticides. The rotational strategy has been widely adopted by growers and is applied to ca 85% of the acreage.

For the Department of Agriculture and Agri-Food, Government of Canada, © Minister of Public Works and Government Services Canada 2003. Published for SCI by John Wiley & Sons, Ltd.

Keywords: Grapholita molesta; rotation of insecticides; resistance management

1 INTRODUCTION
The integrated pest management program for the control of insects and mites introduced to fruit growers in Ontario, Canada in the mid-1970s was successful for ca 20 years. The program used pheromone-trap-capture data to time applications of organophosphorus (OP) insecticides (phosmet, azinphos-methyl) to control the hatch of first instar larvae of the Oriental fruit moth, Grapholita molesta (Busck).1 Phosmet and azinphos-methyl were used in this program because the native predator Amblyseius fallacis (Garman), which maintained European red mite, Panonychus ulmi (Koch) populations below economic damage thresholds, had developed resistance to these compounds.2,3 Thus, the occurrence of resistance to phosmet and azinphos-methyl in G. molesta was a serious threat to the tender fruit industry in the Niagara Peninsula of Ontario, Canada.3–6 Because there was no cross-resistance between OPs and pyrethroids in G. molesta, peach growers were advised to switch to the latter insecticides.5,6 However, pyrethroid insecticides also kill many beneficial insects, and reliance on a program of repeated applications of pyrethroids might accelerate the onset of resistance. Therefore, screening for potential alternatives to pyrethroids was initiated, and data indicated that resistance...
to azinphos-methyl in *G. molesta* did not extend to chlorpyrifos or acephate. Field trials indicated that, while neither of these OP insecticides was as effective as a pyrethroid (deltamethrin), either compound could be useful in a resistance management strategy based on rotation of different groups of insecticides.

The development of resistance countermeasures, and a successful resistance management strategy, depend on the nature, frequency and evolution of resistance mechanisms in field populations of pest insects. Mechanisms of resistance to OP insecticides in *G. molesta* were via both enhanced detoxification by esterase and target site insensitivity, so resistance management strategies using synergists [piperonyl butoxide (PBO) or S,S,S-tri-n-butyl phosphorotrithioate (DEF)] may not be successful. We investigated these factors in previous studies and used the information to test resistance management strategies that conserved the efficacy of all classes of insecticides used to control *G. molesta*.

We report here the results of comparative studies on the trends and distribution of frequencies of resistance, as well as the assessment of fruit damage under different resistance management programs. The sustainability of the programs and the need for alternative strategies are discussed.

### 2 EXPERIMENTAL METHODS

#### 2.1 Insecticides

We used technical grade samples of insecticides (≥98% purity), as supplied by the manufacturers, to monitor for resistance in field populations of *G. molesta*. These were OP (azinphos-methyl, malathion), carbamate (carbofuran) and pyrethroid (cypermethrin) insecticides. They were purchased from the Chem Service, West Chester, PA. Commercial formulations of OP, [chlorpyrifos 500 g kg⁻¹ WP (Lorsban, DowElanco Canada Inc, Markham, Ontario), phosmet 500 g kg⁻¹ WP (Imidan, Zeneca Agro, Stoney Creek, Ontario)] and pyrethroid [deltamethrin 50 g liter⁻¹ EC (Decis, Hoechst, Regina, Saskatchewan)] insecticides were used in commercial orchards.

To assess the effectiveness of the resistance management strategies, changes in resistance frequencies were monitored during the 1996–1999 peach growing seasons. Adult males of *G. molesta* were collected from pheromone-baited-traps each morning from different orchard sites in the Niagara Peninsula. Moths were brought to the laboratory and fed overnight from a cotton pad soaked in 10% sugar solution. These moths were exposed to the residues of insecticides in the glass vials to determine the frequencies of resistance. The diagnostic concentrations used in our monitoring program were 0.1 µg per vial for carbofuran, 0.5 µg per vial for malathion and 2.5 µg per vial for cypermethrin. At the diagnostic concentrations all susceptible individuals were killed, and only resistant phenotypes survived. These concentrations were chosen because they maximize differences in responses of susceptible and resistant individuals to the insecticides tested. The diagnostic concentrations separated resistant strains from susceptible ones. There were 125–350 moths tested per generation at each site under each treatment regime.

#### 2.2 Insecticide bioassays

Adult moths were exposed to insecticides using the vial test described by Kanga and Plapp. Glass scintillation vials (20 ml) were treated with 0.5 ml solutions of insecticides in acetone. The vials were rolled until the acetone had evaporated and the insecticides coated all inner surfaces. Vials treated with acetone only were used as controls. Two to three moths were placed in each vial and held at room temperature (22°C ± 1°C, 60% RH, and 16:8 h light:dark); mortality was determined 24 h after exposure. Adults that were unable to fly a short distance (>1 m) when tossed into the air were considered dead.

#### 2.3 Field applications of insecticides

Various *G. molesta* control strategies were evaluated over 4 years at the Jordan Station Experimental Farm (JSEF) of Agriculture and Agri-Food Canada, and in commercial orchards. Different insecticide treatment regimes were tested in four experimental units or blocks. The treatment applied to each experimental unit was unchanged throughout the 4 years of the study. In the experimental units 1 and 2, OP insecticides were used for treatment 1 and pyrethroids for treatment 2. Treatments 1 and 2 were conducted on 0.5 ha of peach (*Prunus persica* (L) Batsch) cv Loring (3 years old in 1996) and on 0.6 ha of cv Loring or Elberta (12 years old in 1996) with trees spaced 6 × 4.3 m² apart, respectively. Each treatment was applied four times a year for the 4 years of the study. In the experimental unit 3, treatment 3 was applied on 0.6 ha of Elberta (12 years old in 1996) with trees spaced 6 × 4.3 m² apart. This treatment consisted of the sequential use of cyclodiene, OP and pyrethroid insecticides (applied twice per year). An apple plot (12 years old) separated experimental units 1 and 2; these sites were 500 m apart. Experimental unit 3 was located 1.5 km away from units 1 and 2. Additional treatments were conducted in two commercial orchards (10 ha, 12 years old) nearby JSEF and in six other commercial orchards across the Niagara Peninsula. The treatments in all commercial orchards consisted of the sequential use of OP and pyrethroid insecticides (applied twice per year). In all experimental units at JSEF the insecticides were applied in 840 liters water ha⁻¹ with a Rittenhouse model 326 air-blast sprayer (Rittenhouse Sprayers, Jordan Station, Ontario) against 1st instar larvae of each of the three to four generations of the *G. molesta* per year. The standard rates used for OP insecticides
were 2.2 kg AI ha\(^{-1}\) for phosmet, 2 kg AI ha\(^{-1}\) for azinphos-methyl, and 0.01 kg AI ha\(^{-1}\) for chlorpyrifos. Pyrethroid insecticides were used at 1.7 kg AI ha\(^{-1}\) for deltamethrin, and 0.07 kg AI ha\(^{-1}\) for cypermethrin. The cyclodiene, endosulfan, was applied at the rate of 2 kg AI ha\(^{-1}\).

Changes in frequencies of resistance to insecticides in field-collected G. molesta, and the percentage of fruit damage were used to assess the effectiveness of each treatment regime in the experimental unit. The frequency of resistance in G. molesta populations to carbofuran (carbamate) and to malathion (organophosphate) were highly correlated (\(r^2 > 0.94\)). Carbamate insecticides were not used in the program but, because of the high correlation, and because carbamate insecticides provided better discrimination among G. molesta phenotypes than did organophosphorus insecticides, we used the carbamate, carbofuran, to monitor for resistance to both classes of insecticide. Consequently, we discontinued the use of malathion to monitor for resistance to OP insecticides at the end of 1996 fruit growing season (see Fig 1). Percentage of fruit damage was determined at harvest from 100–140 of the ripest fruit collected at first pick from each of five rows in each plot. The fruits were examined for surface feeding damage by larvae of G. molesta, and 50% of the fruit were cut open to determine internal feeding that was not visible on the surface.

2.4 Statistical analyses

Data on the percentage survival were arcsine-transformed and analysed using a repeated-measure analysis (PROC Mixed).\(^{11,12}\) Treatments were modeled as a fixed effect; date, and date-by-treatment interactions were modeled as random effects. Unlike the commercial orchards, the chemical treatments at the JSEF site were conducted in a single experimental unit. Therefore, to satisfy the assumptions of the analysis, the years (4) and generations (3–4 per year) were used to provide the replications for the treatment effects, and the treatments (4) and generations provided the replications to test the fixed effects of the year. Similarly, the treatments and years provided the replications to test the fixed effects of the generations. Percentage fruit damage values were arcsine-transformed and analyzed by a general linear model procedure.\(^{12}\) Mean values were separated by Fisher’s protected least significant difference (LSD).\(^{12}\) Percentage survival of adult males exposed to diagnostic concentrations of insecticides was plotted against each generation of G. molesta under each treatment regime for clarity. Because of the size and the location of the commercial orchards across the Niagara Peninsula, daily monitoring of resistance in adult G. molesta was possible only in commercial orchards near the JSEF for the 4 years of the study. Therefore, for the other six commercial orchards tested, data are presented as an annual evolution of resistance at each site.

3 RESULTS AND DISCUSSION

3.1 Evolution of resistance in Grapholita molesta to organophosphorus insecticides under treatment regime 1

The percentage survival of each generation of G. molesta males to diagnostic concentrations (frequency of moths resistant to a given concentration because all susceptible individuals were killed at that concentration) of carbofuran (carbamate), malathion (OP), and cypermethrin (pyrethroid) insecticides in the experimental unit 1 is shown in Fig 1. Initially, 78% of the moths were resistant to carbamate, 72% to OP and 30% to pyrethroid insecticides. The patterns of resistance in G. molesta varied as the seasons progressed. Resistance to the three insecticides decreased by the end of the season in 1996 to 32%, 30% and 0%, respectively. The levels of resistance to OP and pyrethroid insecticides increased substantially by the end of the season in 1997 (75% and 10% survival to OP and pyrethroid insecticides, respectively). In 1998, resistance to OP and pyrethroid insecticides decreased in early season, peaked in mid-season, and declined slightly again by the end of the season (44% survival to OP, 7% survival to pyrethroid insecticides). In 1999, resistance to OP insecticides peaked in the second generation (60% survival) and decreased in the third and fourth generations (18% survival). Conversely, the frequency of resistance to pyrethroids decreased in the first two generations, increased slightly in the third generation, and declined at the end of the season (3% survival). However, even though each generation of OFM was treated with OP insecticides over 4 years, resistance to these declined from 75% in 1996 to 20%

Figure 1. Effect of repeated applications of organophosphorus insecticides on the evolution of resistance as indicated by the percentage survival (± SE) of several generations of Grapholita molesta collected at Jordan Station Experimental Farm. Moths were exposed to diagnostic concentrations of carbofuran (0.1 µg), malathion (0.5 µg) or cypermethrin (2.5 µg) in glass vials from 1996 (1–96 to 3–96) to 1999 (1–99 to 4–99). Because of the high correlation (\(r^2 > 0.94\)) in the frequency of resistance in G. molesta between carbamate and OP insecticides and that carbamates provided better discrimination among G. molesta phenotypes than OPs, we discontinued the use of malathion after 1996, and monitored for resistance with carbofuran to both insecticide classes in subsequent years. Between 125 and 350 moths were tested per generation.
in 1999. Resistance to pyrethroids also decreased from 30% to 3%.

### 3.2 Evolution of resistance in *Grapholita molesta* to pyrethroid insecticides under treatment regime 2

The frequency of resistance to OP and pyrethroid insecticides in the experimental unit 2 decreased by the end of the season in 1996 (21% and 5% resistance to OP and pyrethroid insecticides, respectively) (Fig 2). Organophosphate resistance increased to 53% in 1997. The levels of resistance to organophosphates decreased to 25% in the first two generations of 1998, but increased to 46% in the third generation, then declined to 23% in the fourth generation. Survival of adult males to pyrethroids in 1998 was the lowest in the first and second generations, but increased slightly to 13.5% in the last two generations. Resistance to organophosphates in 1999 increased in the first and second generations and declined to 12% from the third to the fourth generations. Pyrethroid resistance increased only slightly to 5.9% from the first to the fourth generation in 1999.

### 3.3 Evolution of resistance to insecticides in *Grapholita molesta* under the treatment regime 3

Resistance to OP and pyrethroid insecticides in the experimental unit 3 (Fig 3) decreased by the end of the season in 1996 (55% to 20% resistance to OP, and 30% to 0% resistance to pyrethroid insecticides) and in 1997 (33% to 8.6% resistance to OP, and 5% to 0% resistance to pyrethroid insecticides), and continued to decline in 1998 (29% to 19% resistance to OP, and 15% to 10% resistance for pyrethroid insecticides). In 1999, resistance to both insecticides increased slightly in the early season, but declined again in late season. The overall resistance frequency to organophosphates declined from 55% to 14% and resistance to pyrethroids decreased from 30% to 10%.

### 3.4 Evolution of resistance in *Grapholita molesta* to insecticides in two commercial orchards located in Jordan, Ontario

Resistance in *G. molesta* under the treatment regime 4 was monitored in two commercial orchards nearby the Jordan Station Experimental Farm. The percentage survival of OFM to OP insecticides increased slightly from 50% to 60% by the end of the 1997 season (Fig 4). Frequencies of resistance declined in early season to 20%, peaked in the third generation (50%), and decreased in late season to 22%. During the 1999 peach growing season, levels of resistance increased in the second generation of *G. molesta* (35%) and
declined in all others generations. The overall levels of resistance to organophosphates decreased from 60% to 12% in the last three years. Resistance to pyrethroids decreased from 18% to 5% in 1997 and slightly increased to 20% in 1998. The frequencies of resistance to pyrethroids in the four generations of *G. molesta* fluctuated around 20% in 1999. A consistent trend toward decreased resistance to organophosphates occurred in these commercial orchards from 1997 to 1999. Resistance to pyrethroids in *G. molesta* remained mostly unchanged or increased slightly in the last two generations of 1999.

### 3.5 Evolution of resistance to insecticides in *Grapholita molesta* by Year in six different commercial orchards across the Niagara Peninsula

Resistance in *G. molesta* under the treatment regime 4 to OPs varied by year between sites in commercial orchards over 4 years in the Niagara Peninsula (Fig 5). With the exception of the sites at Virgil and Niagara-on-the-Lake-1, ca 21% of resistant individuals were present in all commercial sites tested in 1996. Except for Niagara-on-the-Lake-1, the levels of resistance decreased in all commercial orchards tested in 1997 relative to year 1996. In 1998, resistance increased in all sites compared to that in 1997, except Virgil. Relative to year 1998, resistance to organophosphates increased slightly only in moths collected at the Niagara-on-the-Lake-2 site (35%) in 1999. There were only low levels of resistance detected in moths collected in Niagara-on-the-Lake-1 or the Virgil site from 1996 to 1999.

Analysis of results on the percentage of survival by year for the six different sites indicated that resistance to pyrethroids in *G. molesta* also varied significantly between sites in commercial orchards (Fig 6). The highest levels of resistance in 1996 were found in moths collected in Virgil and St Catharines (28% and 26% survival, respectively). The frequency of resistance decreased in all sites in 1997, increased in 1998 to the highest levels of 33%, found in moths collected in St Catharines, and declined again in all sites in 1999. In the 1999 collections, moths from St Catharines were the most resistant (10% survival).

### 3.6 Efficacy of different treatment regimes on the evolution of resistance in *Grapholita molesta*

Analysis of data (Table 1) on the responses of field collected moths to OP insecticides indicated significant differences between treatment regimes 

\[ F = 3.75; df = 3, 11; P = 0.0225 \]

years

\[ F = 5.15; df = 3, 11; P = 0.0063 \]

and generations tested

\[ F = 3.61; df = 3, 12; P = 0.0414 \]

The date effect (a measure of within-plot variability over time) and the date-by-treatment interactions were not significant 

\[ P > 0.05 \]

Resistance to OPs varied significantly over time with the highest levels in 1996 (Table 1). Overall, treatments 3 and 4 provided significantly lower levels of OP resistance over the 4 years of the study, and the fourth generation of *G. molesta* was the most susceptible to insecticides.

Resistance to pyrethroids showed different patterns from that to OP insecticides (Table 2). The data indicated significant differences between treatment regimes 

\[ F = 5.13; df = 3, 11; P = 0.0064 \]

years

\[ F = 3.08; df = 3, 11; P = 0.0442 \]

but not between generations 

\[ F = 0.68; df = 3, 12; P = 0.5739 \]

The date effect and the date-by-treatment interactions were not significant 

\[ P > 0.05 \]

These results indicated that the frequencies of resistance to pyrethroid insecticides were significantly different under each treatment.
Figure 7. Assessment of fruit damage by Grapholita molesta under treatment regimes T1–T3 as described above at the Jordan Experimental Station Farm. Bars within a group having the same letter are not significantly different [P > 0.05; Fisher’s protected LSD tests].

Table 1. Evolution of resistance in Grapholita molesta to organophosphorus insecticides under different treatment regimes from 1996 to 1999a,b

<table>
<thead>
<tr>
<th>Treatment regime</th>
<th>Year</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (± SE)</td>
<td>29.81 (± 0.07)</td>
<td>30.09 (± 0.06)</td>
</tr>
<tr>
<td></td>
<td>28.83 (± 0.05)</td>
<td>35.33 (± 0.06)</td>
</tr>
<tr>
<td></td>
<td>22.88 (± 0.04)</td>
<td>34.97 (± 0.06)</td>
</tr>
</tbody>
</table>

a Means in row with the same letter are not significantly different [P > 0.05, Fisher’s protected LSD tests].
b Values are percentage of survival of adult Grapholita molesta to diagnostic concentrations of insecticides (0.1 µg per vial of carbofuran, 0.5 µg per vial of malathion) under each treatment regime.

Table 2. Evolution of resistance in Grapholita molesta to pyrethroid insecticides under different treatment regimes from 1996 to 1999a,b

<table>
<thead>
<tr>
<th>Treatment regime</th>
<th>Year</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (± SE)</td>
<td>3.90 (± 0.01)</td>
<td>11.52 (± 0.03)</td>
</tr>
<tr>
<td></td>
<td>8.45 (± 0.03)</td>
<td>7.73 (± 0.01)</td>
</tr>
<tr>
<td></td>
<td>7.33 (± 0.02)</td>
<td>9.06 (± 0.02)</td>
</tr>
</tbody>
</table>

a Means in row with the same letter are not significantly different [P > 0.05, Fisher’s protected LSD tests].
b Values are percentage of survival of adult Grapholita molesta to diagnostic concentrations of insecticides (2.5 µg per vial of cypermethrin) under each treatment regime.

regime. Overall, treatment 1 provided significantly lower levels of pyrethroid resistance over the 4 years of the study.

### 3.7 Percentage of fruit damage under different treatment regimes

Comparative assessments of fruit damage under each treatment regime are presented in Fig 7. In 1996, the data indicated that treatments 2 and 3 provided the lowest percentage of fruit damage at harvest. Although fruit damage was higher under treatment 2, there were no significant differences between treatments. In 1997 and 1999, the percentage of fruit damage was similar between treatments 2 and 3; treatment 1 provided the least amount of insect control. In 1998, the percentage fruit damage was also similar for treatments 2 and 3, but treatment 1 was not significantly different from treatment 2. Overall, treatment 3 had the lowest percentage of fruit damage in the experimental plots during the 4 years of the study.

In commercial orchards, the percentage of fruit damage under treatment 4 was 0.73% for 1997, 0.63% for 1998 and 0.54% for 1999. The percentage of fruit damage in commercial orchards appeared to be similar to that recorded under treatment 3 (0.93% for 1997, 1% for 1998, 1.7% for 1999) (Fig 7). Overall, treatments 3 and 4 were the most effective in reducing fruit damage by G. molesta in either experimental plots or commercial orchards.

### 4 CONCLUSIONS

Our data indicated that resistance to OP and carbamate insecticides was present in field populations of *G. molesta* from the Niagara Peninsula of Ontario, and that the moth is also developing resistance to pyrethroid insecticides. The results also indicated that resistance to both types of insecticides declined or remained unchanged under treatment regimes 3 and 4 from 1996 to 1999. The evolution of resistance followed a consistent pattern in all treatment regimes or programs we tested, in that resistance usually increased in early or mid-season and declined in late season, possibly as a result of the cumulative deleterious effects of resistance genes and dilution through interbreeding of susceptible individuals.13,14 The patterns of resistance in the experimental plots at the end of each season suggested that late-season migration played an important role in the spread of resistance both locally and regionally. Consequently, a resistance management program for *G. molesta* (and possibly for other insect pests) should be more successful when implemented on a large scale or (as occurred here) on most of the acreage in the Niagara Peninsula region.

The overall levels of resistance to all insecticides tested varied by generation of *G. molesta*. The frequency of resistance and percentage of fruit damage were significantly influenced by each treatment regime. Overall, the data suggested that insecticide
resistance in *G. molesta* can be managed. Because resistance to insecticides in this insect is both metabolic and target site, the use of metabolic or target site synergists to manage resistance may not work, but insecticides (chlorpyrifos or acephate) which provided negative cross-resistance may be more suitable in this case. Thus, a strategy based on rotation of classes of insecticide has provided the basis for a resistance management strategy. The rotational strategy implemented in commercial orchards has reduced resistance and provided control of *G. molesta* over the past 4 years. However, there were similar results in the evolution of resistance under treatment 3 (three-way-rotation) implemented in experimental plots and treatment 4 (two-way-rotation) implemented in commercial orchards. The frequency of resistance to organophosphates and pyrethroids was similar in both treatment regimes. Chlorpyrifos used in the rotational strategy was registered on a temporary basis and for use on the first generation of *G. molesta* only. Screening for alternatives (spinosad, fenoxycarb, tebufenozide, acephate, diazinon, endosulfan, mixtures of Bt and insecticides) to pyrethroids has been unsuccessful (Kanga et al, unpublished; Pogoda and Pree, unpublished). The unavailability of alternative insecticides for use in the rotational scheme may reduce the long-term sustainability of the resistance management program. A modified mating disruption strategy has been initiated. In this strategy, the first generation of *G. molesta* was treated with chlorpyrifos, and subsequent generations were managed with sex-pheromone. Preliminary results with this alternative strategy were promising. Thus, the mating disruption technique will supplement the resistance management strategy and provide the tree fruit industry with additional tools for *G. molesta* control in the Niagara Peninsula of Ontario.

**ACKNOWLEDGEMENTS**

This work was supported by the Natural Sciences and Engineering Research Council of Canada and the Food Systems 2002 Research Fund from the Province of Ontario, Project No 7151, and the Matching Investment Initiatives of Agriculture and Agri-Food Canada. We would like to thank R Cao, W Allen (Agriculture & Agri-Food Canada, SCPFRC, Vineland Station, Ontario) for reviewing earlier draft of the manuscript. We also thank FW Plapp (University of Arizona), providing useful discussion and review of the manuscript.

**REFERENCES**