Abundance of *Coleomegilla maculata* (Coleoptera: Coccinellidae) in Corn Rootworm-Resistant Cry3Bb1 Maize

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ABSTRACT Lady beetles (Coleoptera: Coccinellidae) are important polyphagous predators in maize, *Zea mays* L., fields. Transgenic Cry3Bb1 maize hybrids express a coleopteran-specific insecticidal protein derived from *Bacillus thuringiensis* (Berliner) subsp. *kumamotoensis* that is targeted at corn rootworm larvae. This study evaluated impacts of Cry3Bb1 protein-expressing maize, tefluthrin-treated maize, and untreated controls on lady beetle abundance at preanthesis, anthesis, and postanthesis maize-developmental periods near Brookings in eastern South Dakota during 2001 and 2002. The dominant lady beetle species captured on Pherocon AM sticky traps was *Coleomegilla maculata* De Geer. It comprised 73.5 and 69.9% of all adult Coccinellidae caught in 2001 and 2002, respectively. Numbers of *C. maculata* captured in Cry3Bb1 maize were not significantly different from those in untreated plots during preanthesis, and adults were more abundant in Cry3Bb1 maize than in tefluthrin-treated and untreated plots during anthesis and postanthesis. Whole-plant sampling confirmed *C. maculata* predominance with the species representing 89.2 and 91.4% of all adult lady beetles observed in 2001 and 2002, respectively. Whole-plant sampling also indicated a lack of negative effects from Cry3Bb1 maize on abundance of lady beetle eggs, larvae, pupae, or adults. Overall, these findings indicate that Cry3Bb1-expressing hybrids are not likely to impose harmful effects on *C. maculata*, a species common to maize production systems in the northern Great Plains. This research further suggests that Cry3Bb1 maize has the potential for conservation of these beneficial coccinellids in maize production systems.

TRANSGENIC MAIZE HYBRIDS HAVE been developed to express selective insecticidal proteins to control important insect pest species. For example, Cry3Bb1-expressing hybrids include genetic material derived from *Bacillus thuringiensis* (Berliner) subsp. *kumamotoensis* into maize, *Zea mays* L., that causes mortality in corn rootworm, *Diabrotica* spp., larvae. According to the U.S. Environmental Protection Agency (U.S. EPA 2001), corn rootworms have been the primary target of more conventional insecticide use than any other maize pest. It is estimated that 4,445 metric tons of insecticide active ingredients were used during the 2001 growing season and applied over 31% of maize fields planted in the United States to control corn rootworms. Therefore, one of the most important potential benefits derived from a transition to transgenic insecticidal hybrids would be to significantly lower the insecticide load on the environment. Decreased grower reliance on neurotoxins would also reduce the risk of applicator exposure to pesticides and could minimize impacts on nontarget organisms (Pimentel and Raven 2000).

However, coleopteran-specific toxins effective against *Diabrotica* larvae could pose a risk to related insect groups such as other Coleoptera. Impact assessments on abundance of other coleopteran species common to maize habitats are warranted. One of the most important of these beneficial insect groups, the lady beetles (Coleoptera: Coccinellidae) often prey on key insect pest species found in maize. Elliott et al. (2002) found that *Coleomegilla maculata lengi* Timberlake was an abundant lady beetle species on maize in eastern South Dakota. Coccinellids use many types of prey (e.g., aphids, phytophagous mites, and other insect larvae) and also forage on pollen (Hodek and Honek 1996). Some coccinellids consume up to 50% of their diet as pollen without suffering adverse developmental effects. Furthermore, various coccinellid species are capable of completing development on a diet consisting solely of maize pollen (Lundgren et al. 2004). Increased densities of *Coleomegilla maculata* De Geer were observed by Cottrell and Yeargan (1998) and Lundgren et al. (2004, 2005) when maize

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pollen was present. Omnivorous feeding behavior could increase the exposure of some maize-inhabiting coccinellid species to the Cry3Bb1 protein. These Cry3Bb1 protein levels have been measured at 30–93 μg per gram of fresh weight pollen and leaf tissues (U.S. EPA 2003). A lower titer was reported for maize roots and ranges from 32 to 66 μg per gram of fresh weight tissues; however, lady beetles would not encounter these belowground maize tissues while foraging. If proven sensitive to this insecticidal protein in transgenic maize, these coccinellids would be at increased risk during and after pollen shed associated with maize anthesis.

Our objectives were to compare the relative abundance of nontarget adult Coccinellidae in transgenic, soil insecticide-treated, and untreated maize during preanthesis, anthesis, and postanthesis periods of plant development. Furthermore, sampling was conducted to ascertain whether life stage-specific (egg, larval, pupal, or adult) impacts on coccinellid abundance occur after deployment of transgenic maize for corn rootworm management.

Materials and Methods

Plots were established in 2001 and 2002 near Brookings, SD, in 44.5-ha fields planted to maize during the preceding year to increase the likelihood of rootworm infestations during the study. Treatments were 1) Force 3G (tefluthrin; Syngenta Crop Protection, Greensboro, NC) at 1.48 kg ([AI])/ha; 2) Cry3Bb1-expressing corn rootworm-resistant maize (YieldGard Rootworm; Monsanto Co., St. Louis, MO); and 3) an untreated control. A nontransgenic near-isoline to the Cry3Bb1 maize hybrid was used in tefluthrin-treated and untreated control plots. All treatment plots were arranged in a modified randomized complete block (RCB) design with four replications in 2001. This modification involved a 201-m exclusion zone established between Cry3Bb1 plants and any other commercial maize hybrid to comply with an Experimental-Use Permit restriction by the United States Environmental Protection Agency and the United States Department of Agriculture. To adhere with this requirement, our Cry3Bb1 maize plots also were restricted to the easternmost side of all replications. No internal exclusion zone was required in 2002, and a more conventional RCB design was used; however, a minimum 201-m buffer zone was again placed around the outer perimeter of the study site. Maize seed from the same maturity groups (114 and 110 d in 2001 and 2002, respectively) was used for all treatments to ensure that anthesis occurred simultaneously in all treatment plots and to avoid possible confounding impacts of maize phenological differences on attractiveness to prey, coccinellid beetles, or other arthropods. Maize was planted on 25 May 2001 and 17 May 2002, respectively, in twelve 1.6-ha plots (125 by 125 m) with 0.76-m row spacing. All plots were established on land that had been in maize production the prior season and with similar cropping history and edaphic characteristics. Soybean, *Glycine max* (L.) Merr. (Roundup-Ready, Monsanto Co., St. Louis, MO), was planted to establish nonmaize borders for treatment separation (19 m) and isolation of the experiment. An exception to this occurred in 2001 when an exclusion zone was used around Cry3Bb1 maize plots as described previously. In 2002, a 19-m soybean buffer was used to separate treatments, and any peripheral maize fields were a minimum of 201 m from the outermost Cry3Bb1 plots.

Cry3Bb1 maize hybrids are not effective in managing other secondary insect pests (e.g., wireworm, white grub, seedcorn beetle); thus, Monsanto Co. markets these rootworm-resistant hybrids with a low rate of an insecticidal seed coat treatment. Imidacloprid (Gaucho 600FS; Bayer CropScience, Research Triangle Park, NC) was applied to maize seed planted in the control and Cry3Bb1 plots at a rate of 0.16 mg ([AI])/kernel to protect plots from potential infestations of secondary pests. This rate of imidacloprid is substantially (ca. eight-fold) lower than the 1.31 mg ([AI])/seed rate of imidacloprid (Prescribe, Gustafson LLC, Plano, TX) that targets corn rootworms and secondary pests. Also, no imidacloprid coating was needed on kernels used in the tefluthrin-treated plots because this insecticide is used to control both corn rootworm larvae and secondary pests.

Sticky Traps. Pherocon AM sticky traps were used to monitor activity of adult coccinellids in maize plots in the following growth periods (Julian dates): 1) preanthesis (185–204), 2) anthesis (205–225), and 3) postanthesis (226–254). Sticky traps were 28 by 23-cm yellow cards coated on one side with a sticking agent for insect capture. Traps were placed ≈1 m above the soil surface, mounted on a 1.8-m steel post, and oriented perpendicular to the direction of maize rows to collect adult flying insects. Sixteen traps were placed within the central hectare of each 1.6-ha plot during the V7 maize developmental stage (Ritchie et al. 1996) at 7 to 8 wk after planting. Traps were arranged as four per quadrant, spaced ≈23 m apart, and each was replaced weekly from early July to mid-September. To promote synchrony among sampling dates, initiation of trap placement in 2002 occurred within a calendar day of the date used in 2001.

Whole-Plant Visual Counts. One hundred maize plants were randomly chosen within the central hectare of each plot for quantifying numbers of coccinellids present. The outer surfaces of plants (leaf whorls, axils, tassels, and ears) were visually inspected on each sampling date for eggs, larvae, pupae, and adults to follow the progression of coccinellid life stages throughout the growing season. This was accomplished by visual inspection of plants from the soil line to their apex. Less-exposed plant structures also were dissected to reveal any concealed beetles. As with sticky trap sampling, whole-plant visual assessments were conducted for nine consecutive weeks beginning in early July and ending in mid-September.

Identification and Voucher Specimens. Adult coccinellids were identified to species using the keys of Gordon (1985). Voucher specimens were prepared and deposited in the Severin-McDaniel Insect Re-

search Collection at South Dakota State University, Brookings, SD.

Statistical Analysis. Categorical data from treatment plots did not conform to a normal distribution; therefore, the χ^2 test was deemed as the most appropriate analytical technique. Totals of coccinellid adults captured on sticky traps were analyzed using the PROC FREQ procedure (SAS Institute 2000) that provided χ^2 statistics for the 3 by 3 (period × treatment) frequency table. In cases where significant differences were detected, further partitioning was therefore justified to assist in determining specific differences among phenological stages or treatments. These same analytical procedures also were used to compare treatment differences associated with whole-plant counts of eggs, larvae, pupae and adults.

Results

Adult Coccinellid Collections. *C. maculata* was the dominant coccinellid species, comprising 73.5 and 69.9% of all lady beetles captured in 2001 and 2002, respectively. Because of the importance of maize pollen as a dietary component for *C. maculata*, data from sticky trap collections were interpreted separately for collection periods before, during and after maize anthesis.

Preanthesis. No deleterious effects of Cry3Bb1 maize on *C. maculata* were observed during preanthesis in 2001, although differences were evident during the same phenological stage in 2002 (χ^2 = 33.46, df = 2, $P < 0.0001$). Significantly more *C. maculata* adults were found in Cry3Bb1 maize than in tefluthrin-treated plots during 2002 (χ^2 = 21.46, df = 1, $P < 0.0001$). Approximately 58.4% more lady beetles were captured in the transgenic maize plots than in the conventional insecticide-treated maize. No differences were observed when *C. maculata* abundance was compared between Cry3Bb1 maize and untreated plots in 2002.

Anthesis. The total *C. maculata* adults captured was significantly different among treatments during anthesis in 2001 (Fig. 1). This was primarily attributable to 39.6 and 52.3% greater numbers of *C. maculata* adults captured in Cry3Bb1 maize than in tefluthrin-treated (χ^2 = 38.82, df = 1, $P < 0.0001$) and untreated plots (χ^2 = 73.25, df = 1, $P < 0.0001$). Tefluthrin-treated plots had 20.9% more adults than untreated plots during 2001 (χ^2 = 5.84, df = 1, P = 0.0157); however, untreated plots had 39.7% greater *C. maculata* beetle abundance than tefluthrin-treated plots during 2002 (χ^2 = 12.88, df = 1, P = 0.0003). Thus, it is unclear whether tefluthrin-treated maize adversely affects *C. maculata* abundance. As observed in 2001, treatments in 2002 had significantly different numbers of *C. maculata* adults during the anthesis period (χ^2 = 57.20, df = 2, $P < 0.0001$). Captures of *C. maculata* from Cry3Bb1 maize were 61.3 and 35.8% significantly greater than from tefluthrin-treated (χ^2 = 55.21, df = 1, $P < 0.0001$) and untreated (χ^2 = 15.91, df = 1, $P < 0.0001$) plots, respectively.

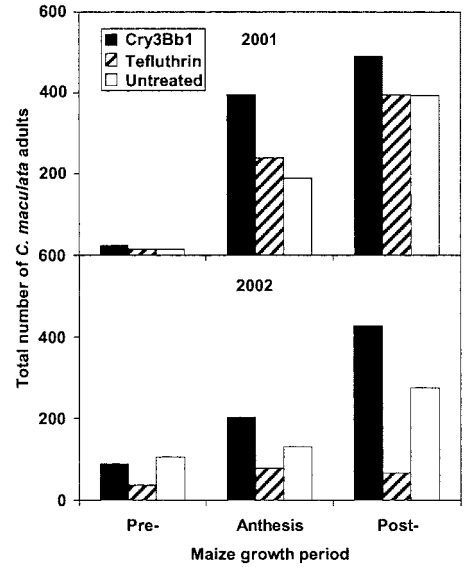

Fig. 1. Total number of *C. maculata* adults captured using sticky traps in maize near Brookings, SD, in 2001 and 2002. 2001 *C. maculata* adults (χ^2 = 23.2, df = 4, $P < 0.0001$). 2002 *C. maculata* adults (χ^2 = 43.3, df = 4, $P < 0.0001$). Sticky trap placement in plots was 1 d later in 2002 than 2001. Sampling periods and Julian dates are preanthesis (185–204), anthesis (205–225), and postanthesis (226–254).

Postanthesis. Significant differences among treatments were detected for *C. maculata* adults in 2001 during the postanthesis period with 19.4% more lady beetles captured in Cry3Bb1 than in tefluthrin-treated plots (χ^2 = 10.20, df = 1, P = 0.0014) and 19.6% more in transgenic maize than in untreated controls (χ^2 = 10.43, df = 1, P = 0.0012). Numbers of *C. maculata* adults captured during postanthesis in 2001 in untreated and tefluthrin-treated plots were not statistically different (Fig. 1). In 2002, adult *C. maculata* were significantly different among treatments during postanthesis (χ^2 = 255.91, df = 2, $P < 0.0001$). Abundance *of C. maculata* adults was 84.3% greater in Cry3Bb1 maize plots than in tefluthrin-treated plots (χ^2 = 263.28, df = 1, $P < 0.0001$), and 35.5% more in Cry3Bb1 than in untreated plots (χ^2 = 32.82, df = 1, $P < 0.0001$). More *C. maculata* also were captured in untreated maize than in tefluthrin-treated plots (χ^2 = 127.35, df = 1, $P < 0.0001$), suggesting a significant risk to *C. maculata* from this conventional soil insecticide, but no measurable effect from the transgenic maize.

Coccinellid Life Stages. Whole-plant samples provided estimates of coccinellid abundance for individual life stages. A high degree of variability existed in abundance with respect to the preanthesis, anthesis, and postanthesis maize developmental periods selected for monitoring.

Eggs. Total numbers of coccinellid eggs varied significantly among treatments during preanthesis in 2001 (Fig. 2). Egg numbers were significantly lower (24.1% less) in Cry3Bb1 maize than in tefluthrin-treated plots (χ^2 = 11.39, df = 1, P = 0.0007), and

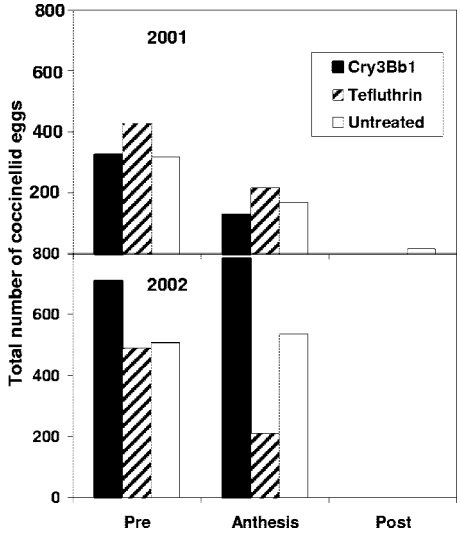

Fig. 2. Total number of coccinellid eggs observed using whole-plant sampling in maize near Brookings, SD, in 2001 and 2002. 2001 coccinellid eggs ($\chi^2 = 36.20$, df = 4, $P < 0.0001$). 2002 coccinellid eggs ($\chi^2 = 106.00$, df = 4, $P < 0.0001$). Species composition of these eggs was not assessed; however, immature lady beetle stages from these plots contributed to an overall 89.2 and 91.4% *C. maculata* adult population as estimated from whole-plant sampling for 2001 and 2002, respectively. Sampling periods by Julian date: preanthesis (185–204), anthesis (205–225), and postanthesis (226–254).

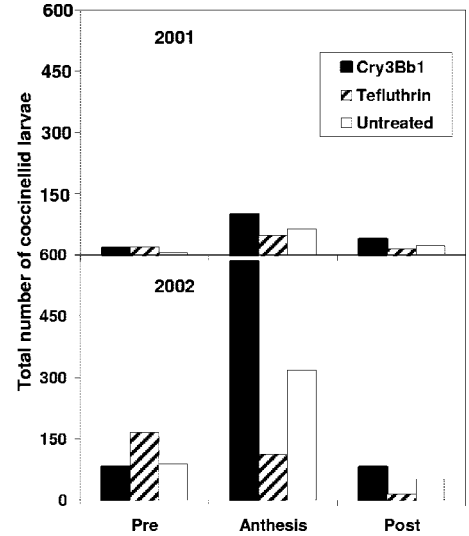

Fig. 3. Total number of coccinellid larvae observed using whole-plant sampling in maize near Brookings, SD, in 2001 and 2002. 2001 coccinellid larvae ($\chi^2 = 13.75$, df = 4, $P = 0.0081$). 2002 coccinellid larvae ($\chi^2 = 256.14$, df = 4, $P < 0.0001$). Species composition of these larvae was not assessed; however, immature lady beetle stages from these plots contributed to an overall 89.2 and 91.4% *C. maculata* adult population as estimated from whole-plant sampling for 2001 and 2002, respectively. Sampling periods by Julian date: preanthesis (185–204), anthesis (205–225), and postanthesis (226–254).

significantly greater (25.9%) in tefluthrin-treated plots than in untreated plots ($\chi^2 = 13.22$, df = 1, $P = 0.0003$). In 2002, egg numbers were significantly different among treatments during preanthesis ($\chi^2 = 52.88$, df = 2, $P < 0.0001$). Egg numbers were significantly greater (31.2 and 28.5%) in Cry3Bb1 maize than in tefluthrin-treated ($\chi^2 = 40.80$, df = 1, $P < 0.0001$) and untreated ($\chi^2 = 33.56$, df = 1, $P < 0.0001$) plots, respectively. No significant difference in numbers of eggs was detected between tefluthrin-treated and untreated maize plots in 2002.

At anthesis in 2001, observed numbers of coccinellid eggs were significantly different among treatments (Fig. 2). Similar to preanthesis, tefluthrin-treated plots had 44.0% more coccinellid eggs than Cry3Bb1 plots during anthesis ($\chi^2 = 22.86$, df = 1, $P < 0.0001$), and more eggs (23.7%) were present in untreated maize than in Cry3Bb1 plots ($\chi^2 = 4.30$, df = 1, $P = 0.0381$). Egg numbers also were significantly different among treatments ($\chi^2 = 323.50$, df = 2, $P < 0.0001$) during anthesis in 2002. Cry3Bb1 maize had 73.1% more eggs ($\chi^2 = 329.82$, df = 1, $P < 0.0001$) than tefluthrin-treated maize during this developmental stage and 31.3% more than untreated plots ($\chi^2 = 45.51$, df = 1, $P < 0.0001$) in 2002.

Extremely low numbers of coccinellid eggs were observed in all treatment plots (Fig. 2) during postanthesis in 2001 and 2002. In total, 14 eggs were observed during the first two sampling dates, and none were detected thereafter. Similarly, no coccinellid eggs were observed during postanthesis in 2002.

Larvae. In 2001, abundance of coccinellid larvae during preanthesis varied significantly between Cry3Bb1 maize (71.4% more abundant) and untreated plots ($\chi^2 = 8.33$, df = 1, $P = 0.0039$) (Fig. 3). Tefluthrin-treated plots sampling found 72.7% more larvae ($\chi^2 = 9.14$, df = 1, $P = 0.0025$) than in untreated maize during preanthesis 2001. No statistical difference was detected in coccinellid larval abundance between Cry3Bb1 and tefluthrin-treated maize during the first year. In 2002, treatment differences were highly different during preanthesis ($\chi^2 = 39.70$, df = 2, $P < 0.0001$). Tefluthrin-treated plots had 51.5% more larvae than Cry3Bb1 maize ($\chi^2 = 29.82$, df = 1, $P < 0.0001$) and had 46.1% more larvae ($\chi^2 = 23.07$, df = 1, $P < 0.0001$) than untreated plots during preanthesis in 2002 (Fig. 3).

During anthesis, significant differences in larval abundance among treatments were observed in 2001 and 2002 (Fig. 3). Numbers of larvae found in Cry3Bb1 maize were 50.5 and 34.6% greater than in tefluthrin-treated ($\chi^2 = 18.23$, df = 1, $P < 0.0001$) and untreated plots ($\chi^2 = 7.73$, df = 1, $P = 0.0054$), respectively. Coccinellid larvae were 80.6 and 45.3% more numerous in Cry3Bb1 maize than in tefluthrin-treated and untreated plots ($\chi^2 = 317.39$, df = 1, $P < 0.0001$ and $\chi^2 = 77.27$, df = 1, $P < 0.0001$, respectively), whereas tefluthrin-treated plots had 64.6% fewer larval coc-

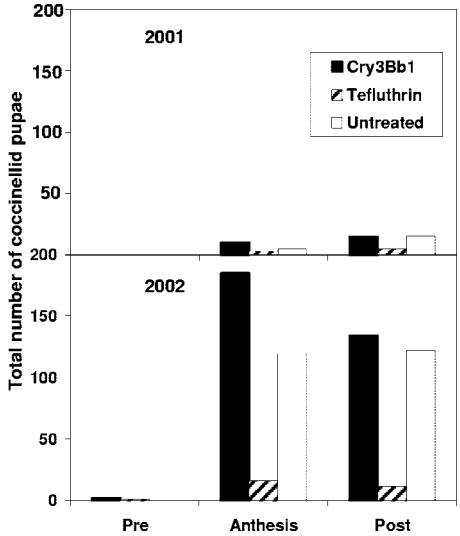

Fig. 4. Total number of coccinellid pupae observed using whole-plant sampling in maize near Brookings, SD, in 2001 and 2002. 2001 coccinellid pupae ($\chi^2 = 5.12$, df = 4, $P = 0.0772$). 2002 coccinellid pupae ($\chi^2 = 10.88$, df = 4, $P = 0.0280$). Species composition of these pupae was not assessed; however, immature lady beetle stages from these plots contributed to an overall 89.2 and 91.4% *C. maculata* adult population as estimated from whole-plant sampling for 2001 and 2002, respectively. Sampling periods by Julian date: preanthesis (185–204), anthesis (205–225), and postanthesis (226–254).

cinellids than the untreated controls ($\chi^2 = 98.23$, df = 1, $P < 0.0001$).

During postanthesis in 2001, larvae were 41.9% more abundant in Cry3Bb1 maize than in untreated plots ($\chi^2 = 4.76$, df = 1, $P = 0.0291$). Significant differences in larval numbers were observed among all treatments during the postanthesis period in 2002 ($\chi^2 = 42.74$, df = 2, $P < 0.0001$). Cry3Bb1 maize had 80.2% more coccinellid larvae in 2002 than tefluthrin-treated plots ($\chi^2 = 43.56$, df = 1, $P < 0.0001$), and 35.8% more larvae were observed in Cry3Bb1 maize than in untreated plots ($\chi^2 = 6.32$, df = 1, $P = 0.0119$). More (69.2%) larvae also were observed in tefluthrin-treated than in untreated plots ($\chi^2 = 19.06$, df = 1, $P < 0.0001$, Fig. 3). Overall, these findings indicate that Cry3Bb1 did not have a negative impact on larval numbers during postanthesis in either year of the study.

Pupae. Numbers of coccinellid pupae observed before the onset of anthesis in 2001 and 2002 were not sufficient to provide meaningful results. No significant differences in pupal numbers during preanthesis were found in either year. Total numbers of pupae differed significantly among treatments during anthesis in 2001 ($\chi^2 = 12.24$, df = 2, $P = 0.0022$) and in 2002 ($\chi^2 = 136.16$, df = 2, $P < 0.0001$). Cry3Bb1 maize had 63.6 and 91.4% more coccinellid pupae than tefluthrin-treated plots during anthesis in 2001 ($\chi^2 = 9.80$, df = 1, $P = 0.0018$) and 2002 ($\chi^2 = 142.10$, df = 1, $P < 0.0001$), respectively. More pupae also were observed in Cry3Bb1 maize than in untreated plots during 2001 (51.5%; $\chi^2 = 5.90$, df = 1, $P = 0.0152$) and 2002 (35.7%; $\chi^2 = 14.33$, df = 1, $P = 0.0002$). Additionally, numbers of pupae in the untreated controls at anthesis were 25% ($\chi^2 = 0.57$, df = 1, $P = 0.4497$) and 86.6% ($\chi^2 = 78.60$, df = 1, $P < 0.0001$) higher than in tefluthrin-treated plots during 2001 and 2002, respectively.

Highly significant differences between pupal densities in Cry3Bb1 maize and tefluthrin-treated plots (Fig. 4) were evident during postanthesis in 2001 (67.3%; $\chi^2 = 16.75$, df = 1, $P < 0.0001$) and 2002 (91.8%; $\chi^2 = 104.34$, df = 1, $P < 0.0001$). Slightly more pupae, 2 and 9% during 2001 and 2002, respectively, were present in Cry3Bb1 maize than in untreated control plots.

Adults. Numbers of coccinellid adults were not significantly different among treatments (Fig. 5) during preanthesis in 2001. Conversely, in 2002, adult abundance was significantly different among all treatments ($\chi^2 = 19.09$, df = 2, $P < 0.0001$). Numbers of adult Coccinellidae observed on Cry3Bb1 maize were 36.5% greater than in tefluthrin-treated plots during preanthesis ($\chi^2 = 18.96$, df = 1, $P < 0.0001$). Adults were also 19.3% more abundant in Cry3Bb1 maize than in untreated plots ($\chi^2 = 4.81$, df = 1, $P = 0.0283$) at preanthesis in 2002.

During anthesis in 2001, significant differences were detected among treatments in the number of adult Coccinellidae (Fig. 5). Tefluthrin-treated plots had 25.8% more adults than Cry3Bb1 maize ($\chi^2 = 10.24$, df = 1, $P = 0.0014$). Also, adults were 26.7% more abundant in untreated plots than in Cry3Bb1 maize ($\chi^2 = 11.08$, df = 1, $P = 0.0009$). In 2002, the number of adults observed during anthesis was again signifi-

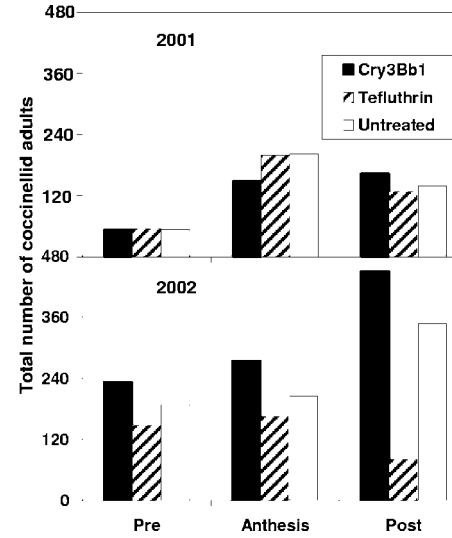

Fig. 5. Total number of coccinellid adults observed using whole-plant sampling in maize near Brookings, SD, in 2001 and 2002. 2001 coccinellid adults ($\chi^2 = 18.00$, df = 4, $P = 0.0012$). 2002 coccinellid adults ($\chi^2 = 91.93$, df = 4, $P < 0.0001$). Approximately 89.2 and 91.4% of these adult coccinellids were *C. maculata* in 2001 and 2002, respectively. Sampling periods by Julian date: preanthesis (185–204), anthesis (205–225), and postanthesis (226–254).

cantly different among treatments ($\chi^2 = 28.87$, df = 2, $P < 0.0001$). Conversely to 2001, transgenic maize plots in 2002 contained 40.0 and 25.5% more adults than that found in tefluthrin-treated or untreated plots ($\chi^2 = 27.50$, df = 1, $P < 0.0001$ and $\chi^2 = 10.21$, df = 1, $P = 0.0014$), respectively.

During the postanthesis period of 2001, numbers of coccinellid adults were not significantly different among treatments (Fig. 5), although 21.1 and 14.2% more adults were observed in Cry3Bb1 maize than tefluthrin-treated and untreated plots, respectively. In 2002, adult coccinellid abundance was significantly different among treatments during postanthesis ($\chi^2 = 248.09$, df = 2, $P < 0.0001$). Eighty-two percent more lady beetles were observed in Cry3Bb1 maize than in tefluthrin-treated plots ($\chi^2 = 256.42$, df = 1, $P < 0.0001$). Adult Coccinellidae also were 22.7% more numerous in Cry3Bb1 maize ($\chi^2 = 13.04$, df = 1, $P = 0.0003$) than in untreated plots. Tefluthrin-treated plots had the lowest adult lady beetle counts and densities were 76.7% lower than in untreated plots ($\chi^2 = 166.18$, df = 1, $P < 0.0001$) during postanthesis in 2002.

Discussion

Impact on Adult *C. maculata* Abundance. Transgenic Cry3Bb1 maize should pose little threat to coccinellids during preanthesis because of the low titer of Cry3Bb1 toxins found in the nonreproductive plant tissues of maize and because most beneficial coccinellids do not readily feed on maize leaf tissues. The observations of adult *C. maculata* on sticky traps and lack of significant differences between numbers of adults captured in Cry3Bb1 maize and untreated plots support this conclusion.

The period of anthesis, when fresh silks and pollen are abundant, represents the time of greatest risk for ingestion of the Cry3Bb1 toxin by susceptible aboveground phytophagous insects. In the current study, significantly more adult *C. maculata* were captured in Cry3Bb1 maize during anthesis than in any other treatment, suggesting that Cry3Bb1 maize does not impart negative effects on this beneficial group during anthesis.

Tefluthrin had mixed effects on adult numbers. Sticky traps in tefluthrin-treated plots had more adult *C. maculata* than those in untreated plots during 2001. In contrast, significantly fewer adult lady beetles were observed in tefluthrin-treated plots than in untreated maize plots during 2002. This could suggests that lower levels of beetle abundance in 2002 could have been caused by our tefluthrin treatment; however, the inconsistency between 2001 and 2002 findings indicates further research is necessary to substantiate this assumption and furthermore, environmental factors existing between the 2 yr of this study also could have produced this disparity in adult lady beetle densities.

Postanthesis sampling was carried out to evaluate late-season and potential chronic effects of Cry3Bb1 beyond the primary period of exposure to pollen and fresh silks. Natural late-season declines in aphid (coccinellid prey) densities that occur as corn leaf tissues senesce may stimulate more lady beetle omnivory, including pollen consumption (Cottrell and Yeargan 1998). This could increase the likelihood of coccinellid exposure to the Cry3Bb1 toxin because of feeding on silk tissues from secondary or tertiary maize ears. However, postanthesis numbers of adult coccinellids were consistently higher in Cry3Bb1 corn than in other treatments, thus suggesting no deleterious effects on *C. maculata.*

Impact on Coccinellid Life Stages. Visual observations on whole plants were used to assess abundance of coccinellid life stages. During preanthesis, Cry3Bb1 maize had equal or greater numbers of coccinellid eggs than the untreated plots. Overall, no deleterious effects from Cry3Bb1 were observed on egg numbers during preanthesis. Likewise, coccinellid larvae also were not negatively impacted by Cry3Bb1 maize during postanthesis. For the most part, coccinellid oviposition in plots had declined by the end of anthesis, so no meaningful egg sampling data were obtainable during postanthesis.

Larval numbers were greater in Cry3Bb1 maize than in untreated plots during preanthesis in 2001, although this pattern was not repeated in 2002. As with the observations on egg numbers, this was possibly due to an absence of other predators in tefluthrin-treated plots. Larvae were most abundant, irrespective of treatment, during the anthesis period. Presumably Cry3Bb1 impacts on larval abundance would have been most evident when pollen and silks were readily available. Larval densities were greater in Cry3Bb1 maize than in untreated and tefluthrin-treated plots in both years and were lowest in tefluthrin-treated plots. Although larval numbers decreased after anthesis both years, more larvae were observed in Cry3Bb1 maize than in tefluthrin-treated and untreated plots. Apparently Cry3Bb1 maize was not detrimental to larvae during the preanthesis or anthesis periods.

Few pupae were observed during preanthesis in any of our plots, but numbers increased considerably during anthesis. Lower numbers of pupae were observed in tefluthrin-treated and untreated plots than in Cry3Bb1 maize during postanthesis. Cry3Bb1 maize also did not negatively impact the total number of adult coccinellids in maize during preanthesis, anthesis, or postanthesis. We conclude that Cry3Bb1 maize was not detrimental to pupae or adult coccinellids. Absence of negative effects was consistent throughout the preanthesis, anthesis, and postanthesis developmental periods of maize. We detected no negative impact on the immature life stages of coccinellids. Our results strongly suggest that Cry3Bb1 maize offers a rootworm management tool that is benign or possibly even beneficial to nontarget coccinellids. Concern over deleterious impacts from the introduction of rootworm-resistant maize hybrids on beneficial insects motivated this research. This study corroborates earlier small-plot field studies by Al-Deeb and Wilde (2003) in demonstrating that Cry3Bb1 maize does not impact abundance of *C. maculata* adults. Similarly,

laboratory research by Duan et al. (2002) and Lundgren and Wiedenmann (2002) found that Cry3Bb1 maize poses minimal risk to *C. maculata* larvae and adults. Hybrids expressing Cry3Bb1 proteins could help to conserve beneficial insects by allowing farmers to decrease reliance on soil insecticides currently used for managing corn rootworm larvae in maize. As a result, increased predator abundance in maize may provide improved natural control of other insect pests in maize production systems. Therefore, our overall conclusion firmly supports the safety of Cry3Bb1-expressing corn rootworm-resistant maize hybrids with respect to nontarget lady beetle abundance.

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