Crop rotation mitigates impacts of corn rootworm resistance to transgenic Bt corn

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Transgenic crops that produce insecticidal proteins from *Bacillus thuringiensis* (Bt) can suppress pests and reduce insecticide sprays, but their efficacy is reduced when pests evolve resistance. Although farmers plant refuges of non-Bt host plants to delay pest resistance, this tactic has not been sufficient against the western corn rootworm, *Diabrotica virgifera virgifera*. In the United States, some populations of this devastating pest have rapidly evolved practical resistance to Cry3 toxins and Cry34/35Ab, the only Bt toxins in commercially available corn that kill rootworms. Here, we analyzed data from 2011 to 2016 on Bt corn fields producing Cry3Bb alone that were severely damaged by this pest in 25 crop-reporting districts of Illinois, Iowa, and Minnesota. The annual mean frequency of these problem fields was 29 fields (range 7 to 70) per million acres of Cry3Bb corn in 2011 to 2013, with a cost of $163 to $227 per damaged acre. The frequency of problem fields declined by 92% in 2014 to 2016 relative to 2011 to 2013 and was negatively associated with rotation of corn with soybean. The effectiveness of corn rotation for mitigating Bt resistance problems did not differ significantly between crop-reporting districts with versus without prevalent rotation-resistant rootworm populations. In some analyses, the frequency of problem fields was positively associated with planting of Cry3 corn and negatively associated with planting of Bt corn producing both a Cry3 toxin and Cry34/35Ab. The results highlight the central role of crop rotation for mitigating impacts of *D. v. virgifera* resistance to Bt corn.

resistance management | resistance mitigation | landscape analysis

Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins can provide significant economic and environmental benefits (1–4) and were planted on more than 100 million hectares worldwide in 2018 (5). The refuge strategy, in which non-Bt host plants growing near Bt crops produce Bt-susceptible insects to mate with Bt-resistant insects surviving on Bt crops, has been widely adopted to slow the evolution of pest resistance to Bt crops and sustain these benefits (6–8). However, evolution of practical resistance to Bt crops, which is field-evolved resistance with practical consequences for pest management, has occurred in at least nine major insect pests in six countries and is accelerating (9–11).

Here we focus on mitigation of the effects of resistance to Bt corn in one of the most destructive crop pests in North America and Europe, a beetle known as the western corn rootworm, *Diabrotica virgifera virgifera* (12, 13). Some populations of this pest in the Midwestern United States have rapidly evolved practical resistance to one or more of the four Bt toxins deployed in Bt corn (10, 13–23), either singly (Cry3Bb, mCry3A, and Cry34/35Ab) or in pairs called "pyramids" (Cry3Bb + Cry34/35Ab, mCry3A + Cry34/35Ab, and mCry3A + eCry3.1Ab) (24). To address this urgent problem, the US Environmental Protection Agency (EPA) mandated supplementation of refuges of corn without rootworm toxins with various tactics to reduce the negative consequences of resistance (i.e., mitigation) and the frequency of resistance alleles (i.e., remediation; refs. 25 and 26).

According to this framework pioneered by companies selling Bt corn seed and corn entomologists (19, 27), the companies must investigate all incidents where farmers report unusually high damage to Bt corn fields. Where root damage exceeds thresholds used as preliminary indicators of Bt resistance (19, 28), the companies must recommend tactics to be implemented by farmers in the following year (26). The primary tactic outlined by EPA is rotation of Bt corn with a nonhost crop such as soybean. Crop rotation has been used for more than a century to reduce *D. v. virgifera* injury to corn, despite adaptation to this tactic in some rootworm populations (19). Secondary tactics include replacing single-toxin Bt corn with dual-toxin Bt corn pyramids, rotation of single-toxin Bt corn producing a Cry3 toxin with Bt corn producing Cry34/35Ab, and increased use of corn without toxins active against rootworms (26).

The EPA framework is supported by the limited data available from previous small-scale field studies (13, 14, 18, 29, 30) and results from simulation models (31, 32). However, the effectiveness of the proposed tactics remains uncertain without comprehensive analyses of field data. For example, rotating the

Significance

The western corn rootworm, a major insect pest in the Midwestern United States, has evolved resistance to genetically engineered corn that produces insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Bt). To evaluate tactics for reducing the damage caused by resistant rootworms, we analyzed field data for 2011 to 2016 from Illinois, Iowa, and Minnesota. The frequency of corn fields with severe rootworm damage was reduced by rotating corn with other crops and by not planting the same type of Bt corn year after year in the same field. These results support the EPA's recommendations to decrease the negative impacts of rootworm resistance to Bt corn by rotating corn with other crops and diversifying the type of Bt corn planted.

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planting of a field between corn and soybean across years is recommended because larvae hatching from eggs laid in a field planted to corn cannot develop on soybean in that field in the subsequent spring. However, previous work has not determined if this crop rotation mitigates the effects of *D. v. virgifera* resistance to Bt corn for populations that have adapted to this tactic by laying eggs in soybean that hatch in the next spring when planting rotates back to corn (19, 30, 32).

We used a long-term, large-scale analysis to evaluate effectiveness of crop rotation and corn diversification for mitigating problems caused by resistance to Bt corn in *D. v. virgifera*. We define problem fields here as: Bt corn fields for which farmers reported greater than expected rootworm damage to Monsanto Company; Monsanto personnel determined that plants from the fields produced the appropriate Bt toxins based on ELISA results; and root damage exceeded nodal injury thresholds consistent with rootworm resistance to Bt corn (19, 28). We analyzed 6 y of data on the frequency of problem fields of Cry3Bb corn (FPF, the number of problem fields per million acres of Cry3Bb corn) across 25 crop-reporting districts (CRDs) (https://www.nass.usda.gov/Charts_and_Maps/Crops_County/boundary_maps/indexgif.php) of Illinois, Iowa, and Minnesota. We focused on these three states because the relatively high number of problem fields there facilitated statistical analyses. For 2011 to 2016, we assessed factors associated with the temporal decline in FPF. For 2011 to 2013, when problem fields were relatively common, we evaluated the association between FPF and the percentage of corn acres rotated to soybean in the previous year (hereafter corn-soybean rotation) as well as acreage of different types of corn. For these years, we also compared the mitigating effects of corn-soybean rotation in CRDs with and without prevalent *D. v. virgifera* resistance to corn rotation to test the hypothesis that resistance to crop rotation reduced its effectiveness for mitigating Bt resistance problems. We compared historical management between problem fields of Cry3Bb corn and all corn fields to assess how farmer practices influenced the frequency of problem fields. Finally, we estimated the cost of damage from *D. v. virgifera* to Cry3Bb corn and the potential economic value of higher than observed use of corn-soybean rotation.

Results

Problem Fields of Cry3Bb and Cry3Bb + Cry34/35Ab Corn. Root node-injury scale (NIS) scores exceeding 1 for Cry3Bb corn or 0.5 for Cry3Bb + Cry34/35Ab corn are often associated with rootworm resistance to the relevant Bt toxins (19, 26, 28). For problem fields, these NIS thresholds were exceeded for 98.9% of Cry3Bb corn fields and 100% of Cry3Bb + Cry34/35Ab corn fields; mean NIS was 1.8 (SE = 0.02) for Cry3Bb corn and 1.4 (0.7) for Cry3Bb + Cry34/35Ab corn. The few problem fields of Cry3Bb corn with a mean NIS score < 1 had an associated SE that overlapped 1. The mean percentage of acres damaged per problem field was 75.8% (SE = 1.1) for Cry3Bb corn and 65.3% (4.6) for Cry3Bb + Cry34/35Ab corn. These results indicate that resistant rootworms were sufficiently abundant in problem fields to cause substantial damage.

Decrease in Rootworm Damage and Shift from Single-Toxin to Dual-Toxin Bt Corn. The total number of problem fields in Illinois, Iowa, and Minnesota during 2011 to 2016 was 18 times higher for corn producing Cry3Bb alone (927) than for corn producing Cry3Bb + Cry34/35Ab (52) (Fig. 1). The millions of acres planted per year decreased for Cry3Bb corn from a mean of 3.6 (SE = 0.4) in 2011 to 2013 to 1.1 (0.2) in 2014 to 2016 (Fig. 2*B*) and concomitantly increased for Cry3Bb + Cry34/35Ab corn from 1.6 (0.3) in 2011 to 2013 to 3.6 (0.2) in 2014 to 2016 (Fig. 3*B*). For both types of Bt corn and all three states, the FPF and percentage of acres with rootworm damage were lower in 2014 to 2016 than in the preceding 2 to 3 y (Figs. 2 and 3).

For Cry3Bb corn, the annual mean FPF fell from 29.2 (SE = 8.5) in 2011 to 2013 to 2.3 (1.0) in 2014 to 2016, a 92% decrease (Fig. 2*C*). The annual mean percentage of Cry3Bb corn acres damaged by rootworms dropped from 0.32% (SE = 0.11) in 2012 to 2013 to 0.018% (0.013) in 2014 to 2016, a 95% decrease (Fig. 2*D*). For Cry3Bb + Cry34/35Ab corn (Fig. 3), damage was lower than for Cry3Bb corn (Fig. 2), but the temporal decrease in damage was similar to Cry3Bb corn. The annual mean FPF for Cry3Bb + Cry34/35Ab corn declined from 2.7 (1.0) in 2011 to 2013 to 0.2 (0.07) in 2014 to 2016, a 93% decrease (Fig. 3*C*). The percentage of Cry3Bb + Cry34/35Ab corn acreage damaged by rootworms fell from 0.023% (0.0085) in 2012 to 2013 to 0.0013% (0.00050) in 2014 to 2016, a 94% decrease (Fig. 3*D*).

Factors Associated with Temporal Decline in FPF. For 2011 to 2016, we analyzed corn-soybean rotation and eight other factors potentially affecting FPF only for Cry3Bb corn because the total of 52 problem fields of Cry3Bb + Cry34/35Ab corn recorded during this period is too small to yield meaningful results (Fig. 3). Because characteristics of the overwintering population from the previous year influence damage to Cry3Bb corn in the current year (see *Methods*), we tested the hypotheses that rotating corn with soybean and planting different corn types during the previous year affected FPF during the current year. Factors included in the analysis were year, state, June precipitation, days with minimum temperature below 0 °F between 1 December and 30 April, percentage of corn-soybean rotation in the previous year, and acreage of four types of corn in the previous year (Cry3 only, Cry34/35Ab only, Cry3 + Cry34/35Ab, and no rootworm-active toxin) (*SI Appendix*, Fig. S1). We included the two weather factors because they have the potential to reduce FPF by decreasing the pest's population size (33, 34).

Variation in FPF was significantly affected by state (P = 0.0025) and year (P = 0.0092) (Covariance analysis for repeated measures, *SI Appendix*, Table S1). The year effect reflects the decrease of FPF in 2014 and the maintenance of a low FPF thereafter, whereas the state effect reflects higher FPF in Iowa than the other two states (*SI Appendix*, Fig. S1). After accounting for these effects, corn-soybean rotation was negatively associated with FPF (P = 0.016). This association was negative in every year (Principal component analysis, *SI Appendix*, Fig. S2), with an overall slope of −0.038 (*SI Appendix*, Table S1). Thus, across CRDs, a 1% increase in corn-soybean rotation was associated with a 3.7% decrease in FPF (see *Methods*). The mean annual percentage of corn-soybean rotation for 2010 to 2015 was 62.6% (SE = 2.2) in Illinois, 59.3% (1.4) in Iowa, and 69.3% (1.6) in Minnesota. In all three states, this percentage decreased from 2010 to 2011, then increased from 2012 to 2015 (Fig. 4). Thus, the decline in FPF from 2013 to 2016 paralleled the increase in corn-soybean rotation from 2012 to 2015.

The two weather factors and four other variables were not significantly associated with FPF (*SI Appendix*, Table S1), although the association between June precipitation and FPF was marginally significant (P = 0.066). However, this association was only negative in 2011 and 2012 (*SI Appendix*, Fig. S2), before the decline in FPF occurred (*SI Appendix*, Fig. S1).

Among-Year Variation in Effects of Corn-Soybean Rotation and Corn Types on FPF. Because most of the problem fields occurred from 2011 to 2013, but relatively few from 2014 to 2016 (Fig. 2*A*), we analyzed spatial variation in FPF for 2011 to 2013 in greater detail. In analyses of individual years, FPF varied significantly among states for each year (Multiple regression, *SI Appendix*, Table S2, $P \leq 0.037$). The percentage of corn-soybean rotation was significantly negatively associated with FPF across the CRDs in 2 of the 3 y (2011, P = 0.0003; 2013, P = 0.027), but the effect of acreage of the different corn types was significant only in 2013. During 2013, FPF was negatively associated with pyramided corn

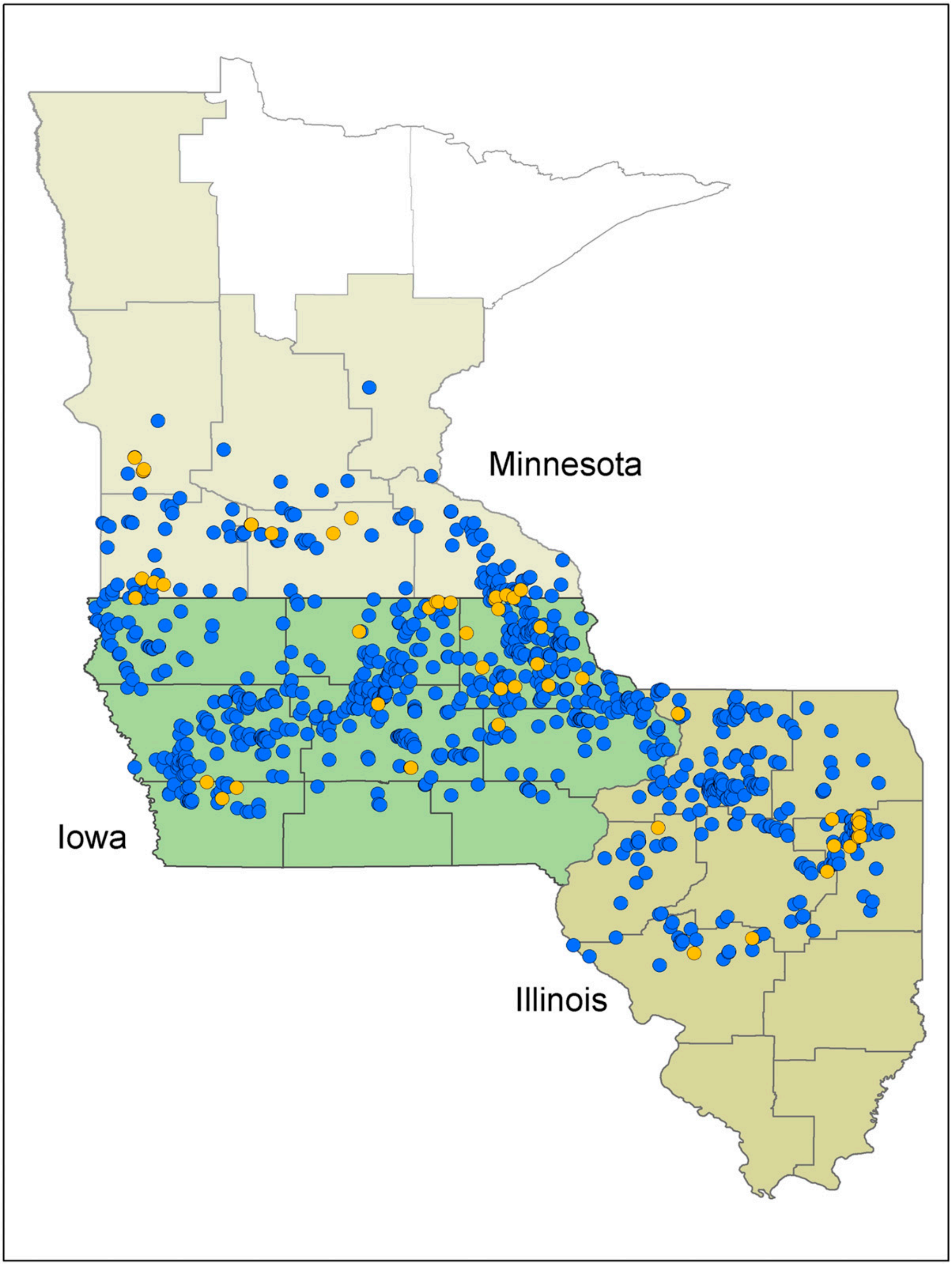

Fig. 1. Problem fields of Cry3Bb corn (blue, total = 927) and Cry3Bb + Cry34/35Ab corn (yellow, total = 52) from 2011 to 2016 in 25 crop-reporting districts (CRDs) analyzed from Illinois, Iowa, and Minnesota. CRDs 2720 and 2730 in Minnesota (white) had no problem fields and were not analyzed because relatively little corn was planted there. We did not plot 13 of the 927 problem fields of Cry3Bb corn because their Global Positioning System coordinates were missing or incorrect.

acreage ($P = 0.042$) and positively associated with single Cry3 toxin corn acreage ($P = 0.027$), but FPF was not significantly associated with Cry34/35Ab corn or corn without rootworm-active Bt toxins (*SI Appendix*, Table S2).

In the pooled analysis for the 3 y, FPF varied significantly among states ($P < 0.0001$) and years ($P = 0.0005$). Across the 3 y, FPF was negatively associated with the percentage of corn-soybean rotation ($P = 0.006$), but not with the acres planted to

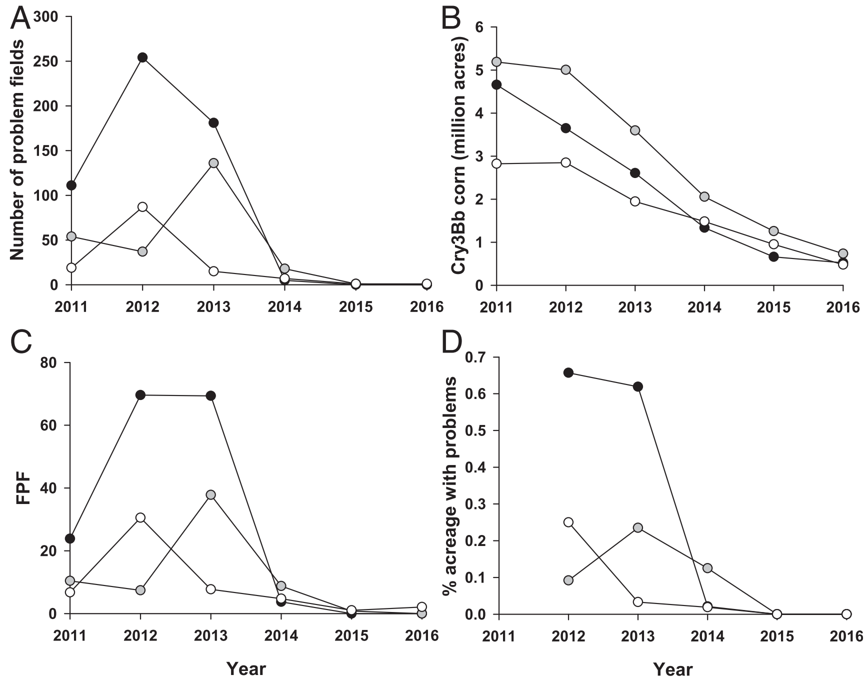

Fig. 2. Cry3Bb corn in Illinois (gray circles), Iowa (black circles), and Minnesota (white circles) from 2011 to 2016. (*A*) Number of problem fields of Cry3Bb corn. (*B*) Cry3Bb corn planted (million acres). (*C*) FPF, problem fields per million acres of Cry3Bb corn. (*D*) Percentage of Cry3Bb corn acres with problems from *D. v. virgifera* (data not available for 2011).

corn producing a single Cry3 toxin, Cry34/35Ab, pyramids, or no rootworm-active Bt toxins (*SI Appendix*, Table S2). Results from these analyses confirm the more consistent across-year effects of corn-soybean rotation relative to acreage of the corn types.

Effect of Resistance to Crop Rotation on Mitigation of Resistance to Cry3Bb Corn. We used partial regression plots for the association between corn-soybean rotation and FPF derived from the above analyses for 2011 to 2013 to test the hypothesis that resistance to crop rotation reduced mitigation of resistance to Cry3Bb corn by corn-soybean rotation. Based on previous reports (12, 35–37), we considered resistance to crop rotation to be prevalent during 2011 to 2013 in seven CRDs of Illinois (1710 to 1770) and CRD 1930 of Iowa (see *Methods*), but not in the other 17 CRDs studied here (Fig. 1). If resistance to crop rotation decreased mitigation of resistance to Cry3Bb by this tactic, then the residuals from the partial regressions should be greater for the eight CRDs with prevalent resistance to crop rotation than for

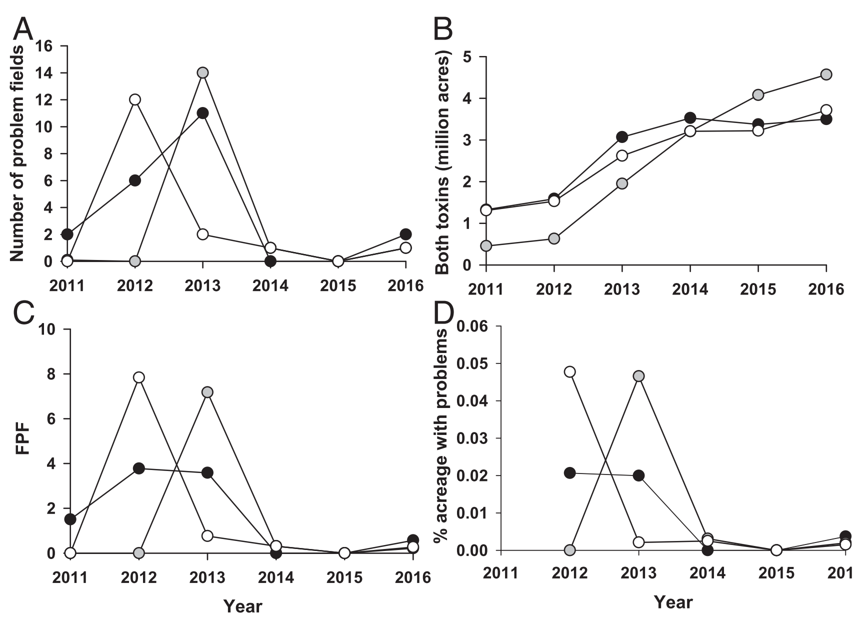

Fig. 3. Cry3Bb + Cry34/35Ab corn in Illinois (gray circles), Iowa (black circles), and Minnesota (white circles) from 2011 to 2016. (*A*) Number of problem fields of Cry3Bb + Cry34/35Ab corn. (*B*) Cry3Bb + Cry34/35Ab corn planted (million acres). (*C*) FPF, problem fields per million acres of Cry3Bb + Cry34/35Ab corn. (*D*) Percentage of Cry3Bb + Cry34/35Ab corn acres with problems from *D. v. virgifera* (data not available for 2011).

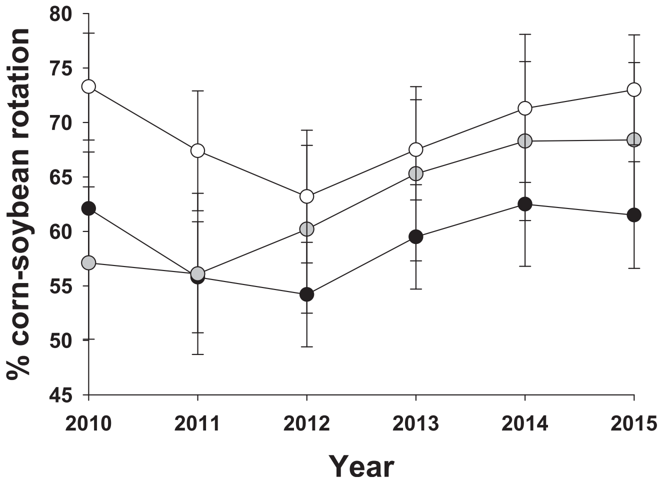

Fig. 4. Mean percentage of corn-soybean rotation in Illinois (gray circles), Iowa (black circles), and Minnesota (white circles) for CRDs considered in covariance analysis of temporal change in FPF (number of problem fields per million acres of Cry3Bb corn). Error bars are SEs.

the 17 CRDs where resistance to rotation was rare or absent. However, the mean residual does not differ significantly between CRDs with and without prevalent resistance to crop rotation in the separate (Two-sample *t* test, *SI Appendix*, Fig. S3) and pooled (Fig. 5) analyses (*SI Appendix*, Table S3, $P \geq 0.064$). Nonetheless, the large positive residual for CRD 1930 of Iowa in 2011 (*SI Appendix*, Table S3 and Fig. S5) suggests resistance to crop rotation may have reduced efficacy of corn-soybean rotation in that case.

Differences in Management History between Problem Fields and all Corn Fields. For each CRD, we compared the management history between the Cry3Bb problem fields and all corn fields to determine if some management practices were more common in problem fields. Because the percentage of Cry3Bb corn acres with rootworm problems was less than 0.7% in all cases (Fig. 2*D*), inclusion of the problem fields as part of all fields in each CRD had little effect. We conducted these analyses for 2012 to 2013 because only these years had a sufficient number of Cry3Bb problem fields (Fig. 2*A* and *SI Appendix*, Tables S4–S6) and information on management history (see *Methods*). For problem fields, we used data from farmer surveys to quantify three aspects of management history during the year problem fields were reported and the three previous years: percentage of corn acres planted with 1) Cry3Bb corn or 2) Cry3Bb + Cry34/35Ab corn, and 3) percentage of years the problem field was rotated to other crops *SI Appendix*, Tables S7–S9). For the CRDs with problem fields, we used AgroTrak and US Department of Agriculture (USDA) CropScape data to quantify corresponding aspects of management history over the same 4-y period: percentage of corn acres planted with 1) Cry3Bb or mCry3A corn or 2) Cry3Bb + Cry34/35Ab or mCry3A + Cry34/35Ab corn, and 3) percentage of corn acres rotated with soybean (*SI Appendix*, Tables S10–S12). For CRDs with problem fields, we focused on the percentage of corn-soybean rotation because remote sensing classification accuracies for crops other than corn and soybean were typically < 50% (see *Methods*).

Confirming the results from the analyses above, rotation of corn with other crops was significantly less common in problem fields than in all corn fields in five of six comparisons (*t* test for paired observations, *SI Appendix*, Table S13). With the exception of Illinois in 2013 ($P = 0.13$), the percentage of corn rotation with all crops in problem fields was significantly lower ($P \leq 0.007$) than the percentage of corn-soybean rotation in all corn fields. The measurement of corn rotation with all other crops in problem fields and of corn rotation only with soybean in all corn fields in CRDs underestimates corn rotation in all corn fields relative to problem fields. Accordingly, these tests are conservative and the results robustly indicate that problem fields were associated with reduced rotation of corn with other crops.

For all years and states, the percentage of Cry3Bb corn in problem fields was significantly higher ($P \leq 0.032$ for each comparison) than the percentage of corn with either Cry3Bb or mCry3Aa in all corn fields (*SI Appendix*, Table S13). The percentage of Cry3Bb corn in problem fields calculated from farmer surveys excludes mCry3A corn for proprietary reasons (see *Methods*) and thus underestimates the percentage of all Cry3 corn in problem fields. Thus, these results are conservative and provide strong evidence that problem fields were associated with frequent use of Cry3Bb corn.

The percentage of Cry3Bb + Cry34/35Ab corn in problem fields was significantly lower than the percentage of pyramided Bt corn in all corn fields in both years in Iowa and Illinois ($P \leq 0.0026$), but not in Minnesota in either year ($P = 0.12$ in 2012 and 0.053 in 2013, *SI Appendix*, Table S13). However, the quantification of only Cry3Bb + Cry34/35Ab corn in problem fields for proprietary reasons (see *Methods*) may underestimate use of all pyramided corn in these fields, favoring overestimation of the difference between problem fields and all fields in a CRD. Thus, the conclusion that growers with problem fields used less pyramided corn than growers without problem fields must be taken with caution.

Economic Impact of Corn-Soybean Rotation. Averaged across states, the estimated cost of yield loss to Cry3Bb corn from *D. v. virgifera* was $227, $209, and $163 per problem acre in 2011, 2012, and 2013, respectively (*SI Appendix*, Table S14). However, because of the low number of problem fields (Fig. 2*A*), losses to *D. v. virgifera* per million acres of Cry3Bb corn averaged across the three states were $0.67 million in 2011, $1.6 million in 2012, and $1.3 million in 2013 (*SI Appendix*, Table S14). For each year, we estimated the value of yield loss to Cry3Bb corn that could have been avoided by increasing corn-soybean rotation in the previous year (*SI Appendix* and Fig. 6). For example, an increase in corn-soybean rotation of 10% in the previous year relative to the observed percentage of rotated corn acres would have reduced losses per million acres of Cry3Bb corn by 31% in each year (Fig. 6).

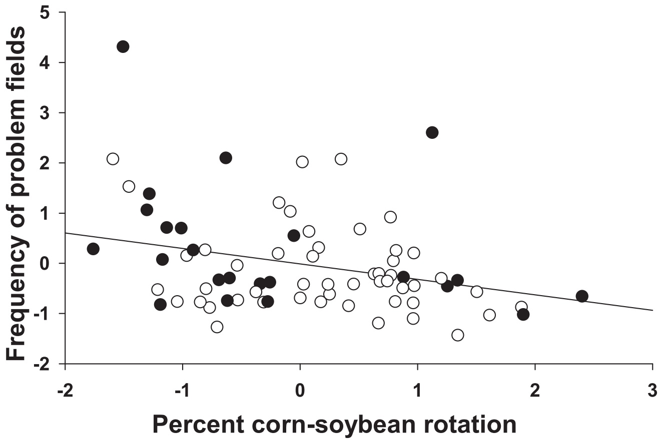

Fig. 5. Partial regression plot for the association between percentage of corn-soybean rotation and FPF (number of problem fields per million acres of Cry3Bb corn) in CRDs for analysis of pooled years (2011 to 2013) reported in *SI Appendix*, Table S3. Studentized Pearson residuals for the percentage of corn-soybean rotation and FPF correspond to crop-reporting districts with (black circles) and without (white circles) prevalent resistance to crop rotation.

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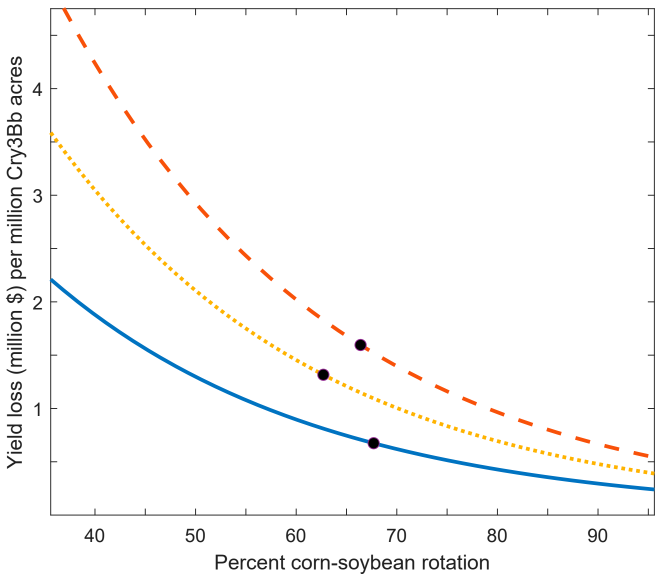

Fig. 6. Potential economic value of higher than observed use of corn-soybean rotation. Circles represent the mean percentage of acres in corn-soybean rotation in the three states. Damage curves for 2011 (solid line), 2012 (dashed line), and 2013 (dotted line) were derived using the association between corn-soybean rotation in the previous year and frequency of problem fields in the current year (*SI Appendix*).

Discussion

Our long-term, large-scale analyses support the EPA's proposed rotation and corn diversification as primary and secondary tactics for remediating problems arising from *D. v. virgifera* resistance to Bt corn (25, 26). In the landscape-based analyses of the 2011 to 2016 data, the percentage of corn-soybean rotation was consistently negatively associated with FPF, and effectiveness of corn-soybean rotation for mitigating resistance problems in fields of Cry3Bb corn did not differ significantly between CRDs with and without prevalent rotation-resistant rootworm populations. Analyses of management history for 2012 to 2013 also support the conclusion that greater use of crop rotation reduced *D. v. virgifera* problems in fields of Cry3Bb corn. By contrast, use of different corn types was not consistently associated with FPF in analyses of the 2011 to 2016 data, although FPF was positively associated with planting of Cry3Bb corn and negatively associated with pyramided corn in 2013. Analyses of management history for 2012 to 2013 indicate that problems in fields of Cry3Bb corn were increased by repeated planting of Cry3Bb corn and potentially reduced by planting of pyramided Bt corn.

Western corn rootworm larvae only complete development on roots of corn and a few grass species (38, 39). In populations that are not resistant to crop rotation, females lay their eggs almost exclusively in corn fields (40, 41). Thus, fields planted to corn after an alternative crop are essentially free of corn rootworm larvae, explaining the resistance-mitigating effects of crop rotation. In populations with prevalent resistance to crop rotation, females lay eggs in fields of different crops (e.g., alfalfa, corn, oat, soybean, wheat), and corn planted after an alternative crop may be vulnerable to rootworm damage (40, 41). The abundance of rotation-resistant adults trapped in soybean fields declined significantly from 1997 to 2015 in Illinois (35, 36). Hypotheses proposed to explain this decline include increased overwintering mortality of larvae caused by higher than normal spring rain, regional suppression of rootworm populations by Bt corn, and widespread insecticide applications in corn and soybean fields (35, 36). The low abundance of rotation-resistant adults during our study could have improved the effectiveness of crop rotation for mitigating damage to fields of Cry3Bb corn. Although our results suggest that elevated June precipitation contributed to reduced rootworm problems in Cry3Bb corn in 2011 and 2012, neither June precipitation nor minimum temperatures below 0 °F between 1 December and 30 April were consistently associated FPF from 2011 to 2016.

Planting Cry3Bb or mCry3A corn in the same field year after year favors evolution of resistance to Cry3 toxins and appearance of excessive root damage (14, 15, 18, 30), which occurs when the abundance of individuals resistant to Cry3 toxins is high (23, 42). Consistent with these studies, our analysis of management history shows that across CRDs of each state, corn with Cry3Bb had been planted more frequently in problem fields of Cry3Bb corn than corn with a Cry3 toxin in all corn fields. Furthermore, in analyses of years when most of the problem fields occurred (2011 to 2013), previous-year use of corn producing a single Cry3 toxin, which is expected to increase the abundance of individuals resistant to Cry3 toxins in overwintering populations, was positively associated with next-year FPF across CRDs of the states in 2013.

Previous-year use of pyramided corn (with Cry34/35Ab and a Cry3 toxin), but not of Cry34/35Ab corn, was also negatively associated with FPF in analysis of the 2013 data. In accord with the low similarity in amino acid sequence of domain II between Cry3 toxins and Cry34/35Ab (43, 44), little or no cross-resistance occurs between them (15, 18, 20, 21, 45). Furthermore, previously published data indicate that during the course of our study, populations of *D. v. virgifera* resistant to Cry3 toxins were more common than those resistant to Cry34/35Ab (22, 23, 29, 45). Thus, previous-year use of pyramided or Cry34/35Ab corn could cause high mortality in individuals resistant to Cry3 toxins and thereby reduce the FPF for Cry3Bb corn in the next year. The significant effect of corn with a Cry3 toxin + Cry34/35Ab but not Cry34/35Ab alone suggests the Cry3 toxins caused some mortality. This could reflect the presence of some individuals not resistant to Cry3 toxins, incomplete resistance to Cry3 toxins (46), or both. Incomplete resistance to Cry3 toxins means that resistant individuals are significantly less susceptible to Cry3 toxins than susceptible individuals, yet they have lower fitness on Cry3 corn than on corn without rootworm-active Bt toxins (46). In fields with previously reported *D. v. virgifera* problems in Cry3Bb corn in Illinois and Iowa, root damage ratings were significantly lower for pyramided than Cry34/35Ab corn (23, 47), and a consistent trend for lower adult emergence in pyramided than Cry34/35Ab corn occurred in three studies (23, 45, 47). In 2012 and 2013, Shrestha et al., (23) sampled 28 *D. v. virgifera* populations in Iowa, primarily from corn fields with a history of rootworm damage to Cry3Bb corn. Although laboratory bioassays revealed that all populations were resistant to Cry3Bb corn relative to susceptible strains, 18% of these populations had significantly lower survival on Cry3Bb corn than non-Bt corn.

Rotation of corn with soybean provides several benefits, including increased yield, control of pests other than corn rootworms, and reduced use of fertilizers (48–51). Furthermore, the economic opportunity costs of rotating corn with soybean depend on the relative prices of these crops (52). During the course of this study, the ratio of corn-to-soybean prices declined by 25% from 2012 to 2013 and the relative price of corn remained low through 2016 (52). Accordingly, the higher net economic return of growing soybean after 2012 (52) likely contributed to the observed increase in corn-soybean rotation from 2011 to 2015. Previous economic modeling has attempted to weigh aggregate yield and revenue benefits of rotation as well as crop price fluctuations. For example, Livingston et al., (50) found that economic benefits are generally maximized over the long-term when farmers plant corn less than 60% of the time, and concluded that as little as a $4 per acre subsidy for corn rotation could be effective for increasing use of this environmentally friendly practice. However, Livingston et al., (50) did not consider the value of reducing damage to corn resulting from resistance to Bt toxins in *D. v. virgifera.* Incorporating results from our study in similar economic models would reinforce the conclusion that there is an economic rationale for incentives to increase crop rotation (8, 50).

Consistent with the expected short-term impacts of crop rotation and abiotic factors on *D. v. virgifera* abundance, in landscape-based analyses we considered short-term impacts of the previous year's tactics on mitigation of problems arising from *D. v. virgifera* resistance to Cry3Bb corn. Because evolution of resistance is affected by the sequence of Bt corn types used in individual fields over several years (14, 15, 18, 30), this may have underestimated the resistance-mitigating potential of the different corn types in landscape-based analyses relative to our analyses of management history that considered the three previous years. Overall, our study indicates that crop rotation is a primary tactic for mitigating problems caused by resistance to Bt corn in *D. v. virgifera*. Together with extensive data documenting practical resistance to Cry3 toxins and Cry34/35Ab (10, 13–23), the results here documenting the distribution of problem fields for both types of corn suggest that sustainability of Bt corn against *D. v. virgifera* is threatened in the Midwestern United States. Increasing crop rotation generally, and adhering to the EPA recommendation of implementing damage-triggered rotation of fields of Bt corn, could be critical for sustaining the economic and environmental benefits provided by rootworm-active Bt corn.

Materials and Methods

Detailed information regarding the study sites, sampling, data collection, calculations, and statistical analyses is provided in the *SI Appendix, Materials and Methods*. The *SI Appendix* also contains *SI Appendix*, Figs. S1–S3 and Tables S1–S14. Data on problem fields of Cry3Bb and Cry3Bb + Cry34/35Ab corn were reported to Monsanto Company by farmers in Illinois, Iowa, and Minnesota during 2011 to 2016. Data for acres of rootworm-active Bt corn and corn without rootworm toxins used in CRDs were obtained from AgroTrak, a commercial data set produced by Kynetec USA. We used the USDA CropScape Cropland Data Layer to calculate the percentage of corn acres rotated to soybean in the year preceding report of problem fields for each CRD and relevant years. We used the National Centers for Environmental Information platform to measure the mean precipitation (inches) between June first and 30th and the mean number of days between 1 December and 30 April with a minimum temperature below 0 °F in CRDs. Data on historical management in problem fields of Cry3Bb corn were obtained from farmer interviews conducted by Monsanto personnel.

Data Availability. Data are available in tables of the *SI Appendix* and Datasets S1 and S2.

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