Sustainable biological control of the soybean aphid: Effects of plant diversity, management practices, and overwintering on *Aphelinus* parasitoids in North America.

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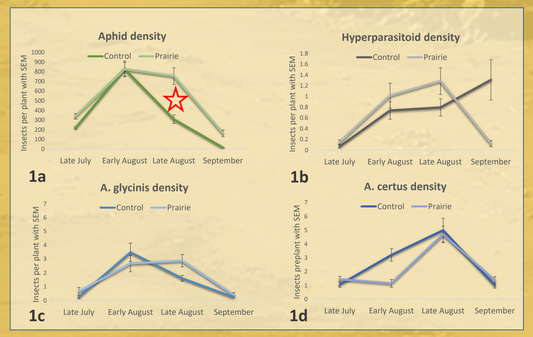
Diversity of plants had little impact on parasitoid control of soybean aphid.

To test the effect of biodiversity on biological control of soybean aphid, we used 3-year-old prairie plots within soybean fields. We compared soybean aphid density and parasitism adjacent to these prairie plots and open soybean fields by 2 parasitoid species – *Aphelinus glycinis* and *A. certus*. Both of these species are Asian in origin, but *A. certus* has 'invaded' on its own and *A. glycinis* is being purposely released for establishment against the soybean aphid. In 2015 we released 120,000 *A. glycinis* over 8 field sites and had excellent recapture of this parasitoid up to 22 mummies per plant after 2 to 4 generations (45 days). Per-plant soybean aphid densities were *higher* adjacent to prairie plots on the third sampling date (Fig 1a) while densities of both parasitoid species were not significantly affected by the presence of adjacent prairie (Figs. 1c, d). Thus, provision of prairie adjacent to soybean fields did not improve biological control of soybean aphid in our study. In addition, we discovered a hyperparasitoid of the *Aphelinus* spp. at our site (a hyperparasitoid is a parasitoid of other parasitoid species) (Fig. 1b). The levels of hyperparasitism (and parasitism) were so low, however, that it is not likely that higher hyperparasitism near prairies is responsible for the overall higher aphid densities adjacent to prairies. *A. glycinis* has not established in North America despite mass releases in 2013 through 2017.

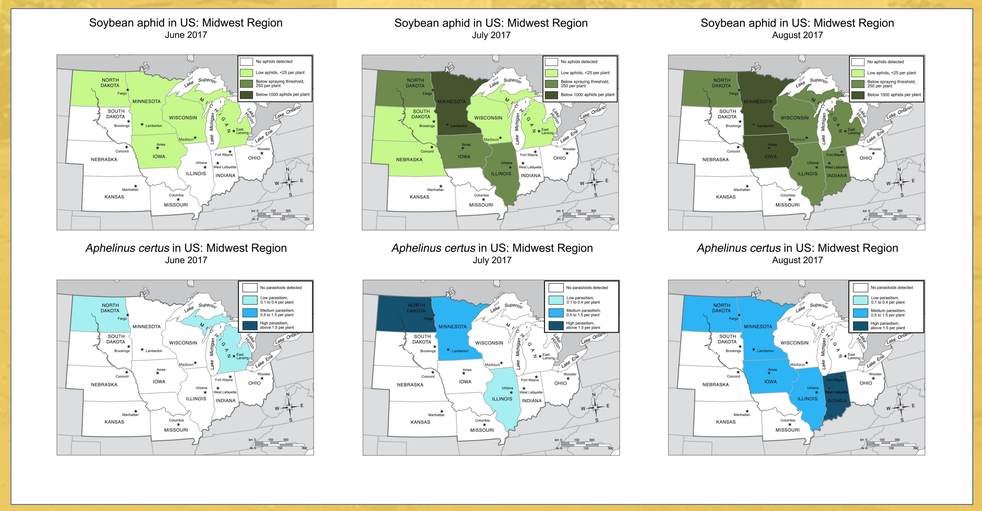
Image 1: *Aphelinus glycinis* female approaching a 2nd instar soybean aphid, credit M. Kaiser.



Biodiversity figures: Insect density by sample date for soybean fields with and without adjacent prairie in Rosemount, MN, 2015. Fields were at least 2 hectares with an experimental plot in the center. The experimental prairie plot was 15 by 15 meters with 15,000 *A. glycinis* released adjacent to the prairie plot; the control fields had no prairie plots, and the data were collected near *A. glycinis* release points within the soybean field.
Red Star: Prairie associated with higher aphid densities, but the only significant difference was in late August.

Regional maps show soybean aphids tend toward the north, and are tracked well by parasitoids.

Regional surveys for soybean aphids and *Aphelinus certus* mummies relied on colleagues across the 12 state Midwest region. Three surveys over the summer of 2017 showed the aphids were found first in the northern tier of states, which also had the greatest aphid numbers as well as the highest density of *Aphelinus*. The darkest green indicates aphid densities above the spraying threshold of 250 aphids per plant.

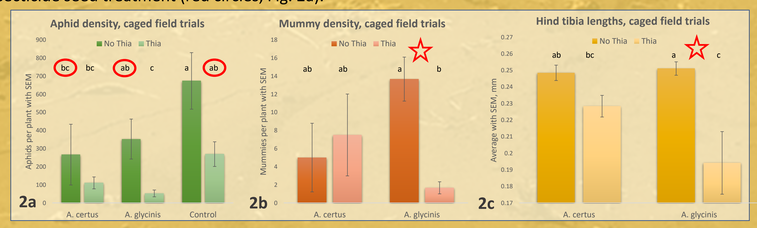


Top maps: States in the North Central U.S. classified as having no aphids, a low level of aphids (< 25/plant), between 25 and the spray threshold of 250/plant or greater than 250 for June, July and August of 2017. Results are based on sampling 20 plants in one field at each starred site.
Bottom maps: States in the North Central U.S. classified by the number of *Aphelinus certus* mummies per plant from the same fields sampled for the top row of maps.

Parasitoids control early season soybean aphids similar to neonicotinoid seed treatments.

Neonicotinoid seed treatments are almost ubiquitous in conventional soybean fields, despite the academic and regulatory advice that they are 1. unneeded and 2. not targeted against the greatest pest, soybean aphid, which usually affects soybean after the neonicotinoid titres are gone from the plants. However, the early season colonization of soybean by both aphids and aphid parasitoids might be reduced by neonicotinoids, limiting biological control. We showed no measurable impact of neonicotinoid seed treatment on *Aphelinus certus*, even in the early season, but an impact of the seed treatment on *A. glycinis*. The impact on *A. glycinis* was both sublethal (smaller body size, Fig. 2c) and lethal (reduced mummy production, see red stars, Fig. 2b). We also showed that in the early season, 4 weeks after planting, *Aphelinus* parasitoids reduced aphid numbers equal to the insecticide seed treatment (red circles, Fig. 2a).

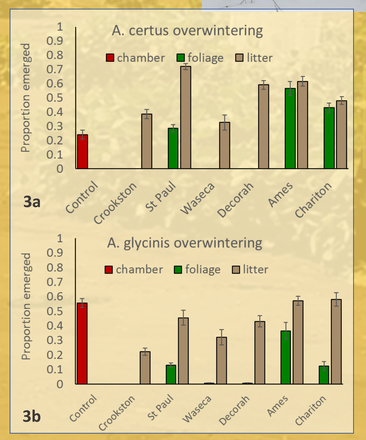
Neonicotinoid figures: Per-plant densities of soybean aphids or *Aphelinus* mummies (2a, b) and *Aphelinus* hind tibia lengths (2c) for different treatment combinations of parasitoid release and thiamethoxam seed treatment from caged soybean plants in the field in St. Paul, MN, 2016. At day 27 after soybean planting, 125 soybean aphids were applied to individual caged plants, and 2 female parasitoids were added on day 28. Hind tibia lengths are a proxy for parasitoid body size and fitness, day 28 to 32 after planting. Note while the experiment produced 42 *A. glycinis* mummies on untreated plants and 19 *A. certus* mummies on treated plants, only 3 *A. glycinis* mummies were found on seed treated plants, all of them male.



Overwintering in Minnesota and Iowa does not limit parasitoids.

Soybean aphid overwinters on European buckthorn, and there is evidence that some soybean aphid parasitoids do as well. Soybean aphids can migrate hundreds of kilometers, and recently parasitized winged soybean aphids exhibit normal flight behavior and thus parasitoids may hitch a ride to buckthorn sites to overwinter. Adult parasitoids may disperse in the Fall as well, and we documented a sharp increase in migration at the end of soybean season when the plants are almost completely senesced. We also found *Aphelinus* mummies on soybean very late in the season suggesting the possibility of overwintering within soybean fields. An examination of leaf litter from soybean fields found both *A. certus* and *A. glycinis* can overwinter in fields.
We evaluated overwintering success by placing diapausing *Aphelinus* mummies in winter conditions along a transect from northern Minnesota to southeastern Iowa. We placed mummies at ground level and at 1 m above ground, at foliar level within buckthorn shrubs. *A. certus* overwintered successfully across all sites in the leaf litter habitat which has a moderated temperature due to snow cover (Fig. 3a). Overwintering success of *A. glycinis* was lower overall and showed a general trend with patterns similar to those of *A. certus* (Fig. 3b).

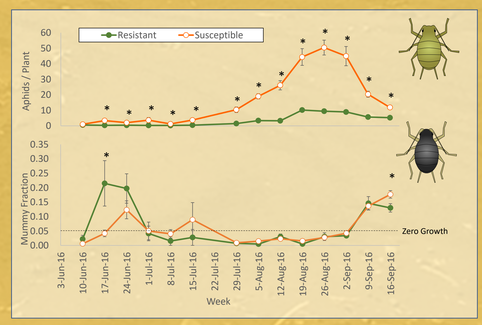
Image 3: Overwintering study site, with mummies protected from predation within mesh sleeves at the foliar (1 m) and leaf litter (ground) levels.



Overwintering figures: Proportion of parasitoid mummies that successfully eclosed as adult parasitoids at 1 m above ground in buckthorn (foliage) and at ground level (leaf litter) across a 1000 km north to south transect from Crookston, MN, to Chariton, IA.

Resistant soybean varieties don't affect parasitoids.

Resistant soybean varieties have been developed through traditional breeding, with *Rag* (Resistance to *Aphis glycines*) strains first reported in 2006, though they have not been widely marketed. We compared parasitism rates in *Rag* soybean with near isoline non-*Rag*. The *Rag* variety reduced aphid numbers throughout the season, and there was no measurable effect on parasitism by *Aphelinus*. 2017 was a year of low aphid densities, which may explain the low recapture of *A. glycinis* despite releases of 2500 wasps in each of 16 soybean plots.



Resistance to *Aphis glycines* figures: Aphid density and mummy fraction (mummies/(mummies + aphids)) on *Rag* and non-*Rag* isoline soybean plants from 10 m square plots in St. Paul, MN, 2016. Zero growth is a rate of parasitism hypothesized to produce aphid control.

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