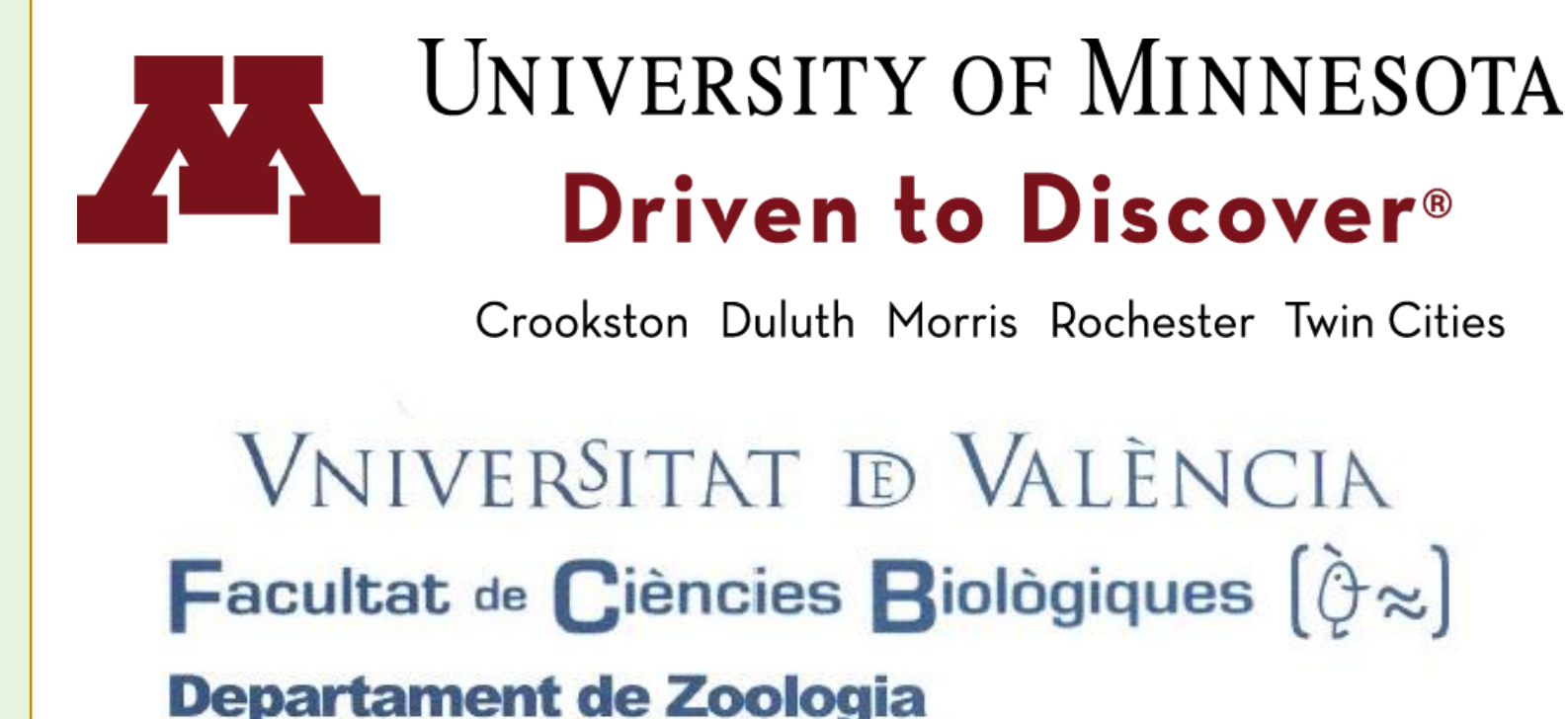


Organisms studied
Insecta: Hymenoptera
Alloxysta brachyptera (Hartig)
Alloxysta brevis (Thomson)
Alloxysta curta Ferrer-Suay
Alloxysta ramulifera (Thomson)
Aphelinus certus Yasnosh
Aphelinus glycinis Hopper and Woolley
Asaphes lucens (Provancher)
Binodoxys communis (Gahan)
Dendrocerus carpenteri (Curtis)
Lysiphlebus testaceipes (Cresson)
Syrphophagus aphidivorus (Mayr)
Insecta: Hemiptera
Aphis glycines Matsumura

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Parasitoids in North American soybean fields: the effect of neonicotinoid seed treatments and hyperparasitism on soybean aphid biological control



Hyperparasitism of soybean aphid parasitoids: four genera, at least seven species

We reared 1,356 hyperparasitoids from 17,345 field-collected parasitoid pupal casings ('mummies') for 5 years in Minnesota. *Syrphophagus aphidivorus* and *Alloxysta brevis* were the most abundant, though 210 *S. aphidivorus* were found in one field in 2017, and *A. brevis* was more widespread across all years.

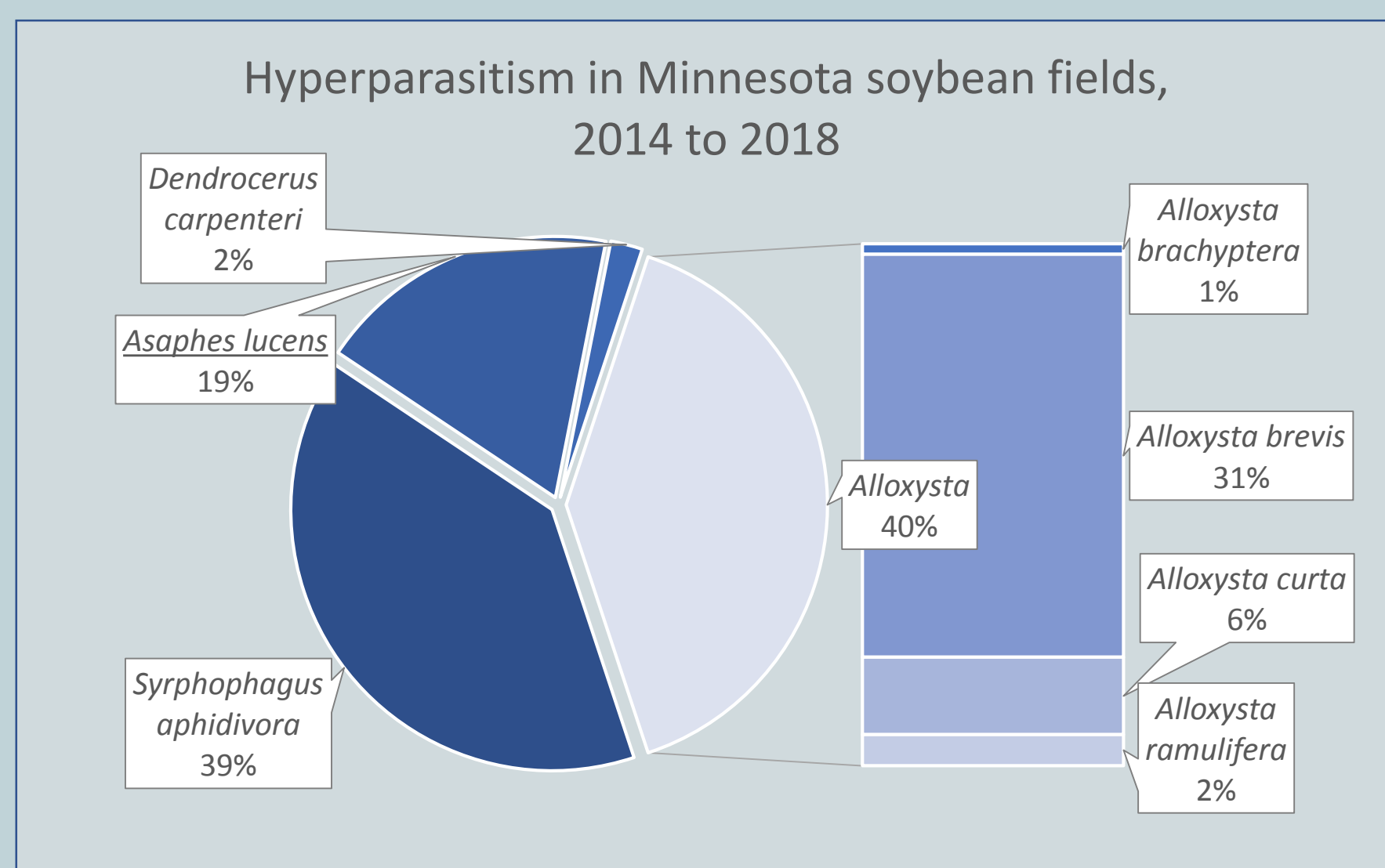


Fig 1. Proportions of hyperparasitoids in Minnesota fields, from field collected mummies. All are Chalcidoidea, *Alloxysta* (Charipidae) being most abundant, followed by the *Syrphophagus aphidivorus* (Encyrtidae), *Asaphes lucens* (Pteromalidae), and *Dendrocerus carpenteri* (Megaspilidae).

Hyperparasitism rates in Minnesota soybean fields, 2014 to 2018.

Hyperparasitism could reduce the biological control of the soybean aphid. We measured a sharp increase in hyperparasitism in 2017, from 7.5% to 17.1%. During these years, soybean aphids were not as prevalent as they were in 2018, when aphid densities were above the action threshold in many Minnesota counties and above the economic injury level in a large number of fields, resulting in foliar insecticide applications.

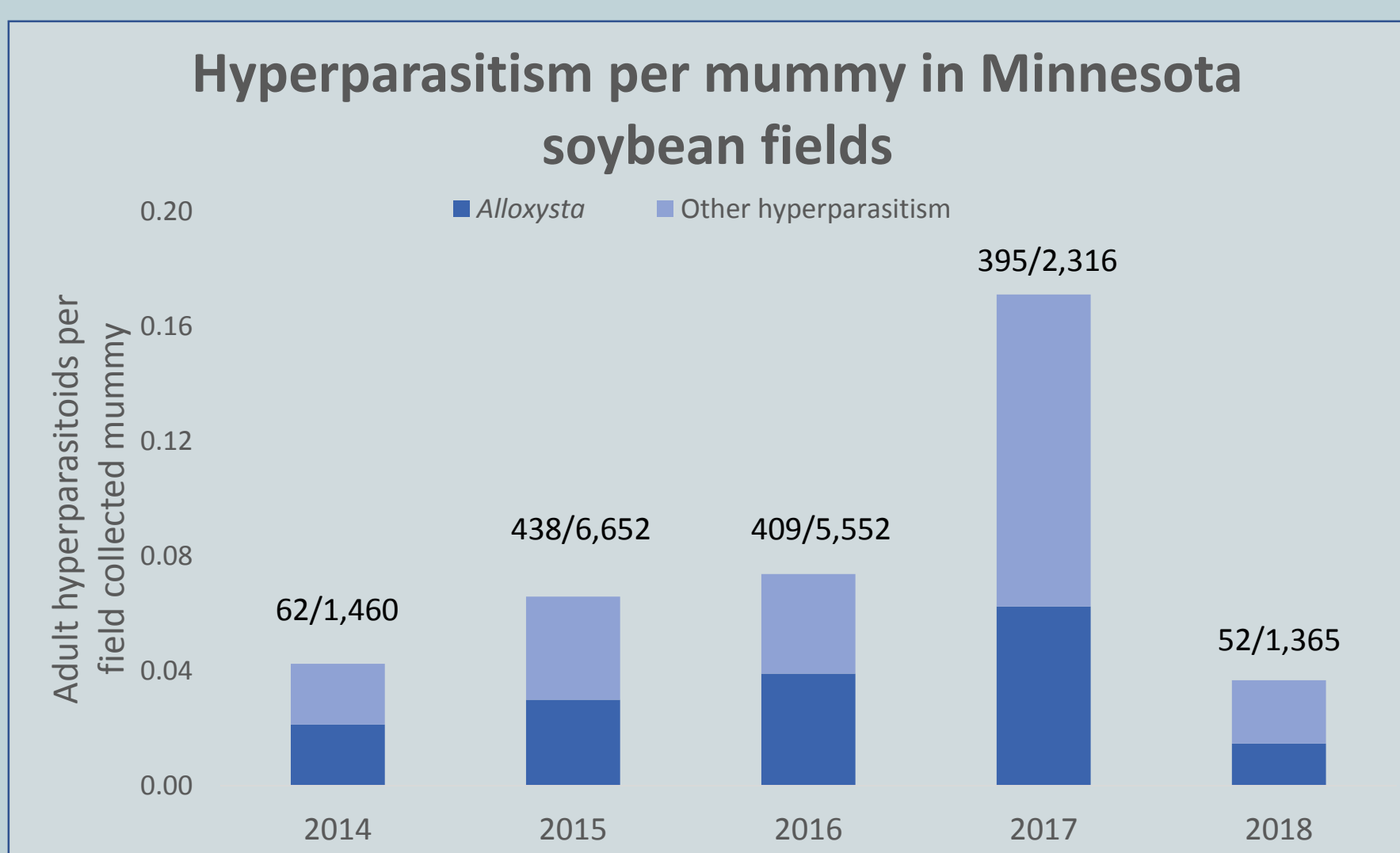


Fig 2. The rates of hyperparasitism were calculated from the number of adult hyperparasitoids emerged from field collected *Aphelinus* mummies. Braconid mummies made up about 0.5 percent of field-collected mummies.

Alloxysta: four species in Minnesota, we discovered that the most common, *Alloxysta brevis*, is thelytokous

In 2017 we observed that Minnesota's 4 species of *Alloxysta* had highly skewed sex ratios, with each species represented by only males (*A. curta* and *A. brachyptera*) or only females (*A. brevis* and *A. ramulifera*). We reviewed a larger sample of specimens from a four year period (Fig. 3) and found female *A. brachyptera* and male *A. brevis*.

In 2018, we monitored field caught *Aphelinus* mummies for emergence of adult *Alloxysta* and found *Alloxysta brevis* to be thelytokous; i.e. females produced daughters from unfertilized eggs (see panel on *Alloxysta reproduction* for details).

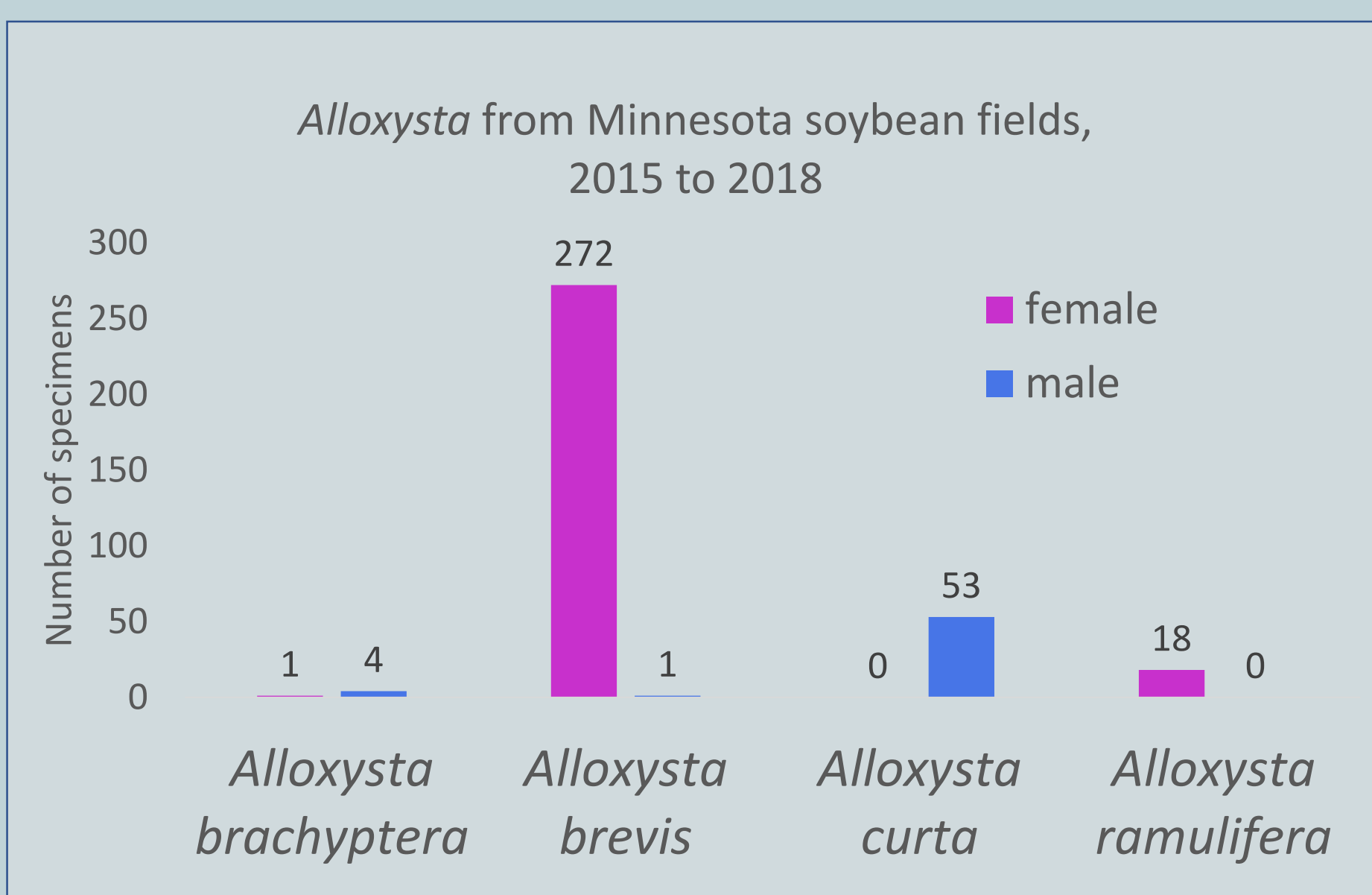


Fig 3. *Alloxysta* from 4 years of sampling were identified to sex and species, resulting in species which were 100% male (*A. curta*) or female (*A. ramulifera*); *A. brevis* was shown to be thelytokous, however 1 male specimen was collected.

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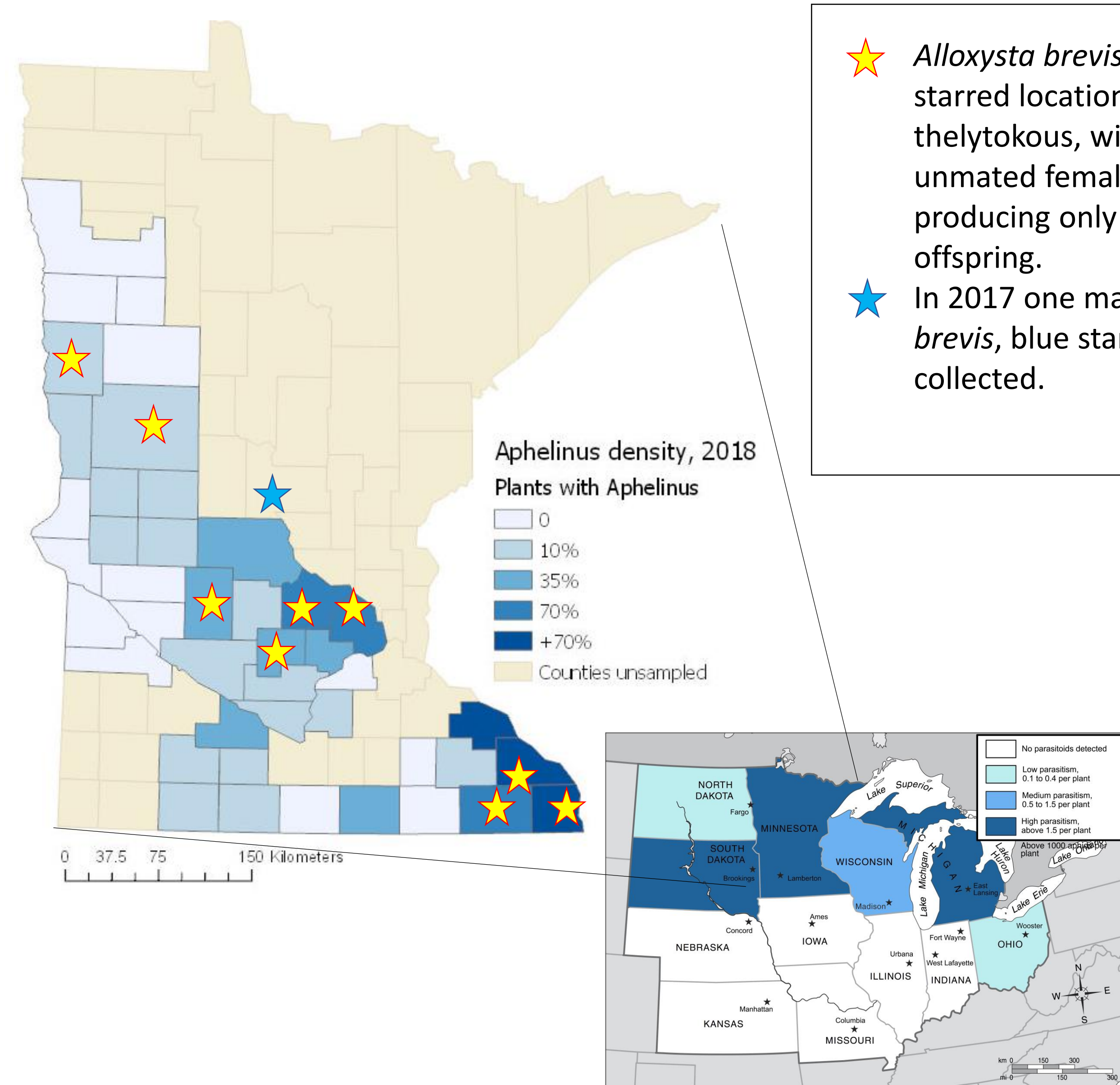
In brief:

- Neonicotinoid seed treatments had no measurable effect on the biological control of soybean aphid by parasitoids.
- Hyperparasitoids were found in soybean fields attacking *A. certus* at rates of 4 to 17 % of mummies emerging as hyperparasitoids
- We found one of the most abundant hyperparasitoids, *Alloxysta brevis*, is thelytokous, i.e., an unmated female adult can produce female offspring.
- We were unable to recover evidence of biological control agents released in Minnesota from 2009 to 2017



Images: 1. Soybean aphid, *Aphis glycines*, on soybean. 2. *Aphelinus* approaching a soybean aphid nymph, preparing to either host-feed or lay an egg. 3. *Aphelinus* mummy is jet black, next to a soybean aphid alate nymph, aphid exuvia in background. 4. *Alloxysta brevis*, cleaning its tarsi. All insects pictured are less than 2mm in length.

Alloxysta brevis: thelytoky shown for the first time in subfamily charipinae



Map 1 Minnesota counties showing proportion of plants with *Aphelinus* mummies, with stars indicating collection sites of *Alloxysta* used in the reproduction assays. Regional map shows *A. certus* densities in soybean fields, summer 2018.

Alloxysta reproduction

Methods: Live unmated adult *Alloxysta* females were offered parasitized aphids in order to produce offspring.

- Field-caught *Aphelinus* mummies were kept in 0.65 ml tubes.
- Upon emergence of an *Alloxysta* adult (parent) the tube was attached to a vial containing parasitized soybean aphids (image 5).
- Mummies were collected after 4 days, and the adults (offspring) which emerged were identified.



Image 5: Arena for hyperparasitism includes emergence tube, and parasitized aphids on a soybean leaflet in damp sand.

Results: 17 unmated *A. brevis* female parents produced 61 female offspring. Maximum hyperparasitism per 48 hours was 13.

Parasitoids control soybean aphids similar to the neonicotinoid seed treatment, thiamethoxam.

We showed no measurable impact of thiamethoxam seed treatment on *Aphelinus certus*, even in the early season when thiamethoxam titres are highest. On young soybean plants, 4 weeks after planting, *Aphelinus* parasitoids reduced aphid numbers equal to the insecticide seed treatment (red circles, Fig. 4).

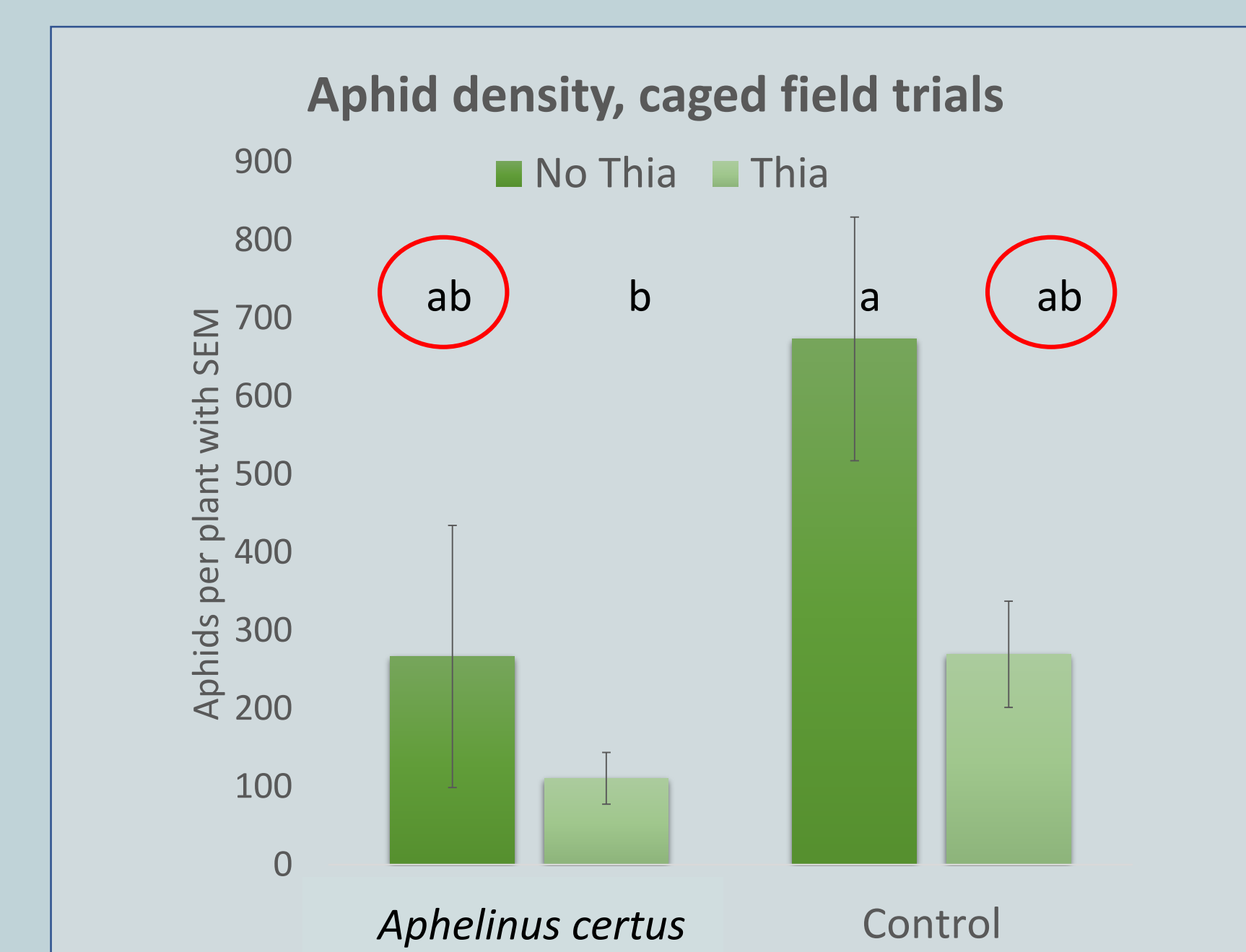


Fig. 4: Per-plant densities of soybean aphids after 12 days, with *A. certus* and/or thiamethoxam seed treatment, from caged soybean plants in the field in St. Paul, MN, 2016. The parasitoids reduced aphid population equal to the effect of the neonicotinoid. *A. certus* was not affected by neonicotinoid, neither in number of offspring nor in fitness as measured by hind tibia length.

Search for aphid biological control agents: *Binodoxys communis* and *Aphelinus glycinis*

Three parasitoids from northern China were permitted for release to control soybean aphid in the USA, and two were mass-released in Minnesota. Yearly from 2009 through 2012 we released about 50,000 *Binodoxys communis*, with some recapture within a few hundred meters during each year but no recapture after 2012.

We released 50,000 to 150,000 *Aphelinus glycinis* each year from 2012 through 2017. Recapture within the year varied widely, from over 1000 recaptured in 2015 to 25 recaptured in 2017.

In 2018 we released no biological control agents, and searched for evidence of establishment using two methods: we collected mummies weekly in sites within 1km of release sites, amounting to 1300 *Aphelinus* and braconid mummies; and we searched the weekly soybean aphid suction trap samples from Rosemount, Minnesota, amounting to 119 *Aphelinus certus* and 12 *Lysiphlebus testaceipes*. We found no *Aphelinus glycinis* and no *Binodoxys communis*.

