CALIFORNIA LEAFY GREENS RESEARCH BOARD
Annual Research Report
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Project Title:
Confirming the resistance breaking and occurrence of *Nasonovia* Nr:1 biotype in the Salinas Valley

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Abstract:
Several PCAs submitted *Nasonovia*-resistant lettuce samples infested with red aphids to the UCCE for proper species identification during the summer and fall of 2018. Aphids from all these samples were identified as the lettuce aphid, *Nasonovia ribisnigri*. After this corroboration, our working hypothesis was that a new biotype for the lettuce aphid was present in the Salinas Valley. Based on reports in other countries, this new biotype can overcome the resistance to *Nasonovia* Nr:0 biotype that has been bred into commercial lettuce cultivars. To validate the occurrence of a new *Nasonovia* biotype, we exposed field-collected lettuce aphids to both *Nasonovia* Nr:0 biotype-resistant and susceptible lettuce varieties in greenhouse tests. Lettuce aphids collected in Gonzales, CA from *Nasonovia* Nr:0 biotype-resistant lettuce suggests the presence of a new *Nasonovia* biotype in Salinas Valley. The breaking of the resistance to *Nasonovia* aphids in lettuce will influence the overall management of this pest. In the short term, growers and PCAs are highly encouraged to scout fields planted with *Nasonovia*-resistant lettuce varieties more often, with the goal of detecting this pest early and executing a suitable control tactic if present.

Objective:
Document the establishment and development of field-collected lettuce aphid on experimental and commercial lettuce aphid-resistant lettuce cultivars.

Procedures:
We originally proposed to screen for the *Nasonovia* resistance breaking in three distinctive lettuce aphid populations collected in Salinas, Gonzales, and Soledad, using whole-plant, no-

choice assays. We transitioned from using whole-plant experimental units, to lettuce disk assays in order to reduce the amount of experimental lettuce material and improve tracking of aphid development (Fig. 1). The new setup for our experimental units consisted of a 1-oz plastic container one third filled with agar and covered with a lid. One lettuce leaf disk was placed inside the container on the solidified agar. Field-collected aphids were brought into the laboratory and held under room conditions overnight. Winged aphids only were selected the next day for no-choice assays, 10 aphids in each container. There were two treatments, each with two variations: lettuce cultivar and aphid origin. Survival of aphids was observed on *Nasonovia*-resistant (Nr:0) lettuce (cv. Fortunas) and susceptible lettuce (cv. Patriot). The two sources of lettuce aphids were a USDA greenhouse culture of biotype Nr:0, reared on *Nasonovia*-susceptible lettuce that did not possess Nr:0 resistance, and field-collected specimens. Thus, there were four treatment combinations, with three containers per treatment for each assay repetition. All experimental containers with aphids were held under ambient room conditions for the duration of the assays. Aphid densities (nymphs and adults) were counted daily for seven consecutive days. There were three repetitions for each assay, where each repetition was started at different dates.

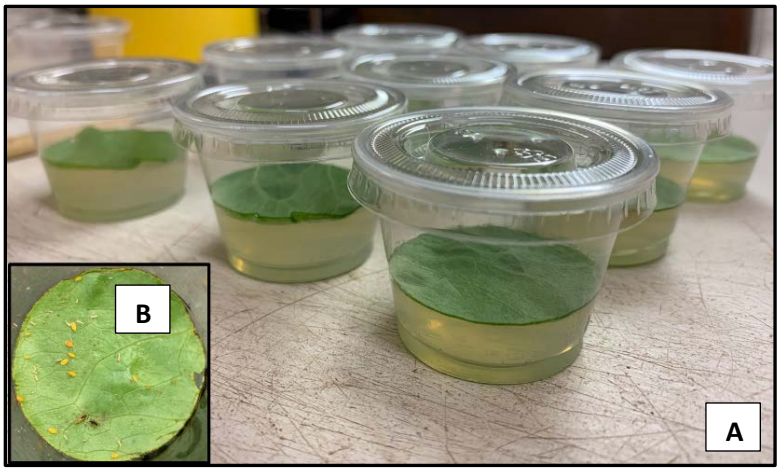

Fig. 1. Lettuce disk no-choice assays. A) A leaf disk was cut and put inside the 1-oz plastic container with agar, then 10 winged aphids were placed onto the leaf disk. B) Aphid nymphs (pink specimens on the disk) started to develop on each disk, and were counted daily. Photo credit: Daniel Hasegawa.

Statistical analysis

Aphid densities were subjected to ANOVA and Tukey's honestly significant difference (HSD) post-hoc test. Data were partitioned by lettuce cultivar. Data from each day within an assay were analyzed separately.

Results:

We sampled lettuce aphid populations from three fields in Soledad, Gonzales and Chualar. The Gonzales colony survived to provide aphids for the assays. The Soledad and Chualar collections failed to survive in culture. Data presented are from the Gonzales colony only.

Performance of aphids on Nr:0-susceptible lettuce (Fig. 2, left panels)
Nr:0 biotype adults from the USDA greenhouse died at a lower rate compared with field-collected aphids. More than half of the population of the Gonzales aphids died by day 2. In contrast, it took four days for more than 50% of the population of the USDA greenhouse aphids (Nr:0 biotype) to die. In parallel, the Nr:0 nymphs reached a high of 30 specimens per container by day 3, compared to 15 nymphs per container from the Gonzales collection.

Performance of aphids on *Nasonovia*-resistant lettuce (Fig. 2, right panels)
Adults aphid from Gonzales had a higher survivorship than Nr:0 adults after 24 h. The survival rate for the Gonzales population was at least 3x higher than Nr:0 adults during the following six days of the assays. A significant contrast between Nr:0 and Gonzales nymph development was also observed. Limited nymph development from Nr:0 specimens was evident after 24 hr, with no more than one nymph per container through the duration of the 7-day assay, while Gonzales nymphs thrived, up to 25 nymphs per container on day 4.

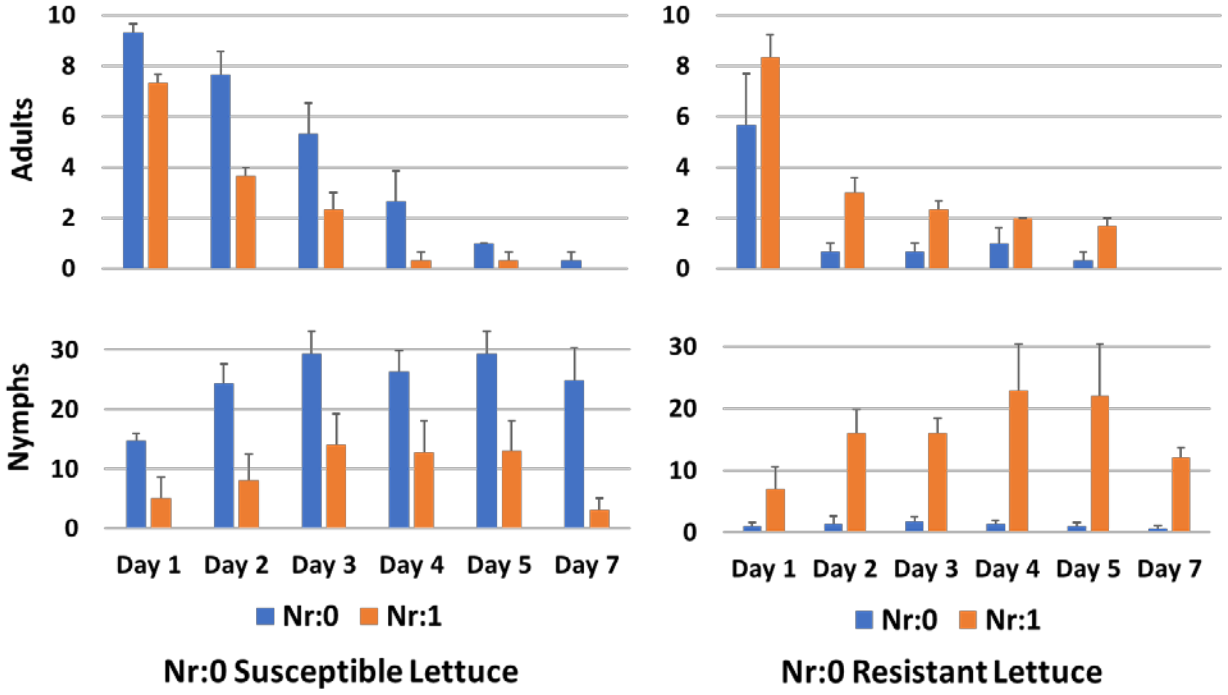

Fig. 2. Performance and development of lettuce aphid adults (top panels) and nymphs (bottom panels) on lettuce cultivars Patriot (left panels, Nr:0-susceptible) and Fortunas (right panels, Nr:0-resistant). Blue bars represent aphids that originated from the USDA greenhouse, considered as the Nr:0 biotype. Orange bars represent field-collected specimens from Gonzales, which based on performance, is considered a new biotype, possibly Nr:1. Bars present the mean (± standard error) number of individuals per container.

Discussion:

Growers and PCAs identified an issue with *Nasonovia*-resistant lettuce cultivars being infested with lettuce aphid during the 2018 growing season. One potential explanation for this issue was presence of a new biotype of the lettuce aphid that overcomes the resistance in commercial lettuce to Nr:0 biotype. The results from this project suggest the presence of this new biotype of the lettuce aphid. The experiments could not determine its identity as the Nr:1 biotype, which was first observed on lettuce in Europe in 2007 (ten Broeke et al., 2013). Additional tests are needed to determine whether or not the Salinas population of Nr:0 resistance-breaking aphids are Nr:1 or a third biotype. Additional collections and testing are needed to confirm how widespread this new lettuce aphid biotype is in the Salinas Valley.

Our data are limited in geographical scope but were consistent with the reports of lettuce aphid development on Nr:0 biotype-resistant lettuce, when specimens were assayed on Nr:0-susceptible and Nr:0-resistant lettuce. Nymphs that originated from field-collected adults were able to thrive on a *Nasonovia* biotype Nr:0-resistant lettuce cultivar. Nr:0 biotype aphids from the USDA greenhouse colony failed to develop on the same *Nasonovia* Nr:0 biotype-resistant lettuce cultivar. An interesting aspect of the Nr:0 biotype resistance-breaking Gonzales sample was its poor performance on a Nr:0-susceptible lettuce variety. Nr:0 biotype developed at a higher rate than the Nr:0 resistance-breaking biotype aphids on Nr:0-susceptible lettuce. Nr:1 biotype survived equally well on Nr:0-resistant and susceptible lettuce (ten Broeke et al., 2013).

In conclusion, our results indicate the presence of a lettuce aphid resistance breaking biotype in Salinas Valley. This new biotype overcame resistance to Nr:0 biotype in currently available *Nasonovia*-resistant lettuce cultivars. This development was first evidenced by establishment of lettuce aphid populations in commercial fields planted with *Nasonovia*-resistant lettuce cultivars in 2018. Based on this project's findings, it is also highly recommended that growers and PCAs scout more frequently their fields planted with *Nasonovia*-resistant lettuce. Early detection of the lettuce aphid on *Nasonovia*-resistance varieties will be key to timely deployment of any necessary control measures.

Acknowledgements:

We thank all the PCAs who submitted lettuce samples to UCCE for aphid identification and let us collect aphids from their fields.

Literature Cited:

ten Broeke, C.J.M., M. Dicke, and J.J.A. van Loon. 2013. Performance and feeding behaviour of two biotypes of the black currant-lettuce aphid, *Nasonovia ribisnigri*, on resistant and susceptible *Lactuca sativa* near-isogenic lines. Bul. Ent. Res. DOI: 10.1017/S0007485312000880