Identifying Onion Fields at Risk of Iris Yellow Spot Virus in New York

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ABSTRACT

Iris yellow spot virus (IYSV) poses a significant threat to dry bulb onion, *Allium cepa* L., production and can lead to substantial yield reductions. IYSV is transmitted by onion thrips, *Thrips tabaci* (Lindeman), but not via seed. Transplanted onion fields have been major early-season sources of IYSV epidemics. As onion thrips tend to disperse short distances, seeded onion fields bordering transplanted onion fields may be at greater risk of IYSV infection than seeded fields isolated from transplanted ones. Additionally, seeded onion fields planted early may be at greater risk of IYSV infection than those seeded later. In a two-year study in New York, we compared IYSV incidence and onion thrips populations in seeded onion fields relative to their proximity to transplanted onion fields. In a second study, we compared IYSV incidence in onion fields with either small or large plants during mid-season. Results showed similar IYSV incidence and onion thrips populations in seeded onion fields regardless of their proximity to transplanted onion fields, while IYSV incidence was over four times greater in large onion plants than in small ones during mid-season. These findings suggest a greater risk of onion thrips-mediated IYSV infection in onion fields with large plants compared with small ones during mid-season and that proximity of seeded fields to transplanted ones is a poor indicator of IYSV risk. Our findings on IYSV spread dynamics provided valuable insights for developing integrated pest and disease management strategies for New York onion growers.

Keywords: epidemiology, *Peribunyaviridae*, risk management, *Thrips tabaci*, *Tospovirus*

Iris yellow spot virus (IYSV) is a member of the *Tospovirus* genus within the *Peribunyaviridae* family. This virus is a significant threat to bulb onion by substantially reducing yield (Pappu et al. 2009). IYSV is transmitted by onion thrips (*Thrips tabaci* Lindeman) and cannot be spread through seed or mechanical means (Gent et al. 2006; Kritzman et al. 2001; Robène-Soustrade et al. 2006). IYSV often causes elongated, necrotic lesions on onion foliage, impairing photosynthetic capacity and resulting in diminished bulb size (Gent et al. 2004). In severe cases, IYSV epidemics can lead to crop losses of up to 100% (Pozzer et al. 1999). Current management of IYSV relies on effective management of the thrips vector using threshold-based insecticide programs (Leach et al. 2019; Nault and Shelton 2010; Regan and Nault 2022).

In recent years, transplant-established onion fields have gained prominence over seeded onion fields in the Elba Muck of New York, which is one of the largest onion production regions in the eastern U.S. Because onions grown in transplanted fields typically grow larger and mature earlier than those in seeded fields, onion bulbs produced in transplanted fields are often more economically valuable, and can be sold sooner than those produced in seeded fields (Ketema et al. 2013). In New York, onions are routinely transplanted by hand from early April through early June, whereas onions are typically seeded from mid-April through mid-May. However, warmer and drier springs have enabled growers to plant onion seeds over a longer period, starting as early as late March and extending into late May. Consequently, there is a wide range in plant sizes among seeded onion fields in this region (B. Nault, personal observation).

Since initial detection in New York in 2006, IYSV has perennially challenged onion production in the Elba muck region, with increasing prevalence and incidence especially in hot, dry years (C. Hoepting, personal observation). Although multiple sources of IYSV exist in this region (Hsu et al. 2011; Smith et al. 2011), past research has suggested that transplanted onion fields are major sources of disease epidemics during the first half of the growing season (Leach et al. 2018). Nearly all onions that are transplanted in the Elba Muck are sourced from the southwestern U.S. (Bartolo et al. 2013), some of which may be infested with onion thrips and infected with IYSV (Hsu et al. 2010; Leach et al. 2018). Additionally, past research showed that onion thrips adults exhibited a preference for colonizing transplanted onion fields over seeded ones early in the season, possibly because onion thrips prefer to colonize large plants in the transplanted fields over small ones in seeded fields (Hsu et al. 2010; Leach et al. 2018).

Onion thrips are more likely to disperse short distances than long distances (Smith et al. 2015, 2016). Smith et al. (2017) reported that onion thrips populations late in the season were higher in non-mature onion fields that bordered mature transplanted onion fields. Presumably, onion thrips migrated from mature transplanted fields into adjacent non-mature fields because the latter was a more suitable host. Smith et al. (2017) also showed that IYSV incidence late in the season was greater along field edges of non-mature onion fields that bordered mature transplanted fields than those not bordering transplanted fields, implying that the proximity of onion fields to transplanted fields might be a site-specific risk factor for disease epidemics. More recently, Komondy et al. (2023) found similar IYSV incidences during the growing season in transplanted and seeded onion fields in the Elba Muck production region in New York. However, neither the proximity of seeded onion fields to transplanted ones nor the phenology of onions in seeded and transplanted fields were addressed in that study. Therefore, onion growers are questioning whether prioritizing onion thrips control in transplanted fields early in the season to curtail IYSV epidemics should be expanded to include seeded fields that border transplanted fields, as well as the relative onion plant size in seeded fields during onion thrips colonization at mid-season.

The goal of this study was to gain a better understanding of site-specific risk of IYSV in the Elba Muck onion production region. The first objective was to compare IYSV incidence and onion thrips populations among transplanted onion fields, seeded onion fields adjacent to transplanted fields, and seeded onion fields that were relatively isolated from transplanted fields. The second objective was to compare IYSV incidence in seeded onion fields that had either "small" or "large" plants during mid-season after adult colonization where plant size was determined by a combination of plant height, neck width, and leaf count measurements. We hypothesized that seeded onion fields bordering transplanted fields would have a higher incidence of IYSV compared with seeded fields isolated from transplanted ones during the second half of the growing season. We also hypothesized that seeded onion fields containing large plants would have a higher IYSV incidence than those containing small plants during mid-season. This study expands on the one recently published on the spatiotemporal patterns of IYSV and onion thrips in transplanted and seeded onion fields by Komondy et al. (2023).

Materials and Methods

Proximity of seeded onion fields to transplanted onion fields on IYSV risk study. Data from Komondy et al. (2023) were used to test new hypotheses in our study. In 2021 and 2022, this study was conducted in the Elba Muck onion production region in New York (43°134' N; 78°099' W). The selection of the Elba Muck was driven by its distinction as one of the primary zones in the eastern U.S. for intensive onion cultivation. Moreover, it has encountered recurring severe epidemics of IYSV (Hsu et al. 2010; Leach et al. 2018; Smith et al. 2015), which have led to substantial economic losses (C. Hoepting, personal observation). The Elba Muck comprises 'Carlisle' and 'Palms' muck soils USDA-NRCS (2004), encompassing nearly 2,200 ha, with approximately half of this area dedicated to annual onion cultivation. Typically, fields are 55 m wide and over 200 m long, often featuring adjacent dirt roads, drainage ditches, and willow hedgerows. Detailed information regarding the locations of all sampled onion fields can be found in Table S1.

In both years, the Elba Muck was divided into four regions that contained one transplanted onion field, one seeded onion field situated adjacent to the transplanted field on at least one side, and one isolated seeded onion field. Therefore, a total of 12 fields were sampled annually (four transplanted fields, four seeded fields bordering transplanted fields, and four isolated seeded fields). Isolated seeded fields were located on average 432 m (range: 203 m to 1,010 m) away from transplanted fields. All transplanted fields were established using "bare-root" (soilless) onion plants imported from the southwestern U.S. All commercially grown onion cultivars in this study lacked resistance traits effective against both thrips and IYSV (Cramer et al. 2017; Damon et al. 2014). Table S1 contains details regarding the cultivars and planting dates, and days to maturity of the onion fields sampled in this study. Transplanted and seeded onion fields were devoid of volunteer onion plants, which can be a source for both IYSV and thrips, shortly after planting because they were either absent or hand weeded.

Onion plants were sampled for onion thrips and IYSV in fields using a grid-based design. In each field, a designated area measuring 55 m wide and 55 m long (0.3 ha) was selected for sampling. This area was overlaid with a 10 by 10 grid, resulting in 100 quadrats, each measuring 5.5 m wide by 5.5 m long. Within each quadrat, four onion plants were chosen via stratified random sampling and the total number of onion thrips adults and larvae were counted and recorded. Segments of each of the four onion plants within the quadrat, including the leaf axil and adjacent leaves (Boateng et al. 2013), were removed and placed in plastic Ziploc® bags (SC

Johnson, Racine, WI) for testing the following day. This four-plant composite sample served as the sampling unit for IYSV testing. The test used to detect IYSV is described below. In 2021, onion plants were sampled on four dates: 14 and 28 June, 12 July, and 2 August. In 2022, sampling occurred on five separate dates: 27 June, 11 July, and 1, 8, 15 August.

Effect of onion plant size on IYSV risk study. Four seeded onion fields containing large plants and four seeded fields containing small plants were tested for IYSV during mid-season on 24 July 2023 (Table S2). The test used to detect IYSV is described below. Sampling during mid-season provided a greater likelihood of detecting differences in IYSV levels between large and small plants than sampling either early in the season when IYSV may be very low (less than 5% incidence) in all fields or late in the season when IYSV may be very high (greater than 80% incidence) in all fields. The sampling process adhered to a similar grid-based approach as the previous experiment. Within each field, the selected sampling area measured 55 m in width and 55 m in length. This area was divided into five transects, each spanning 30 m long, creating 50 sampling quadrats, each measuring 10 m wide by 5.5 m long. In each quadrat, three onion plants were selected, and segments of each onion plant, encompassing the leaf axil and adjacent leaves, were removed and placed in plastic Ziploc® bags (SC Johnson, Racine, WI) for testing the following day. This three-plant composite sample was considered the sampling unit for IYSV testing.

Onion plants within fields are generally uniform in size. Exceptions may occur for plants within the first ~5 m of both the beginning and ending of each row where fertilizer levels and crop protectant concentratons may differ from the rest of the field, as well as low spots in the field that may be wetter than other parts of the field (L. Komondy, personal observation). On 24 July, 2023, seven onion plants were gathered at random within uniform areas of each field and the following measurements were recorded for each plant: (1) plant height, (2) neck width, and (3) the average number of leaves per plant. Disease symptoms were not noted. Plant height was defined as the length of the longest leaf on each plant (cm). Neck width (cm) of each onion plant was measured using calipers, at the base of the onion leaves. Additionally, the number of green leaves on each plant was counted. The mean values for plant height, neck width, and number of leaves per plant were calculated for each field.

Serological assay for IYSV. To enhance the chances of detecting IYSV (Boateng and Schwartz 2013; Hsu et al. 2011; Kritzman et al. 2001), onion leaf tissue was sampled from the inner leaves, including the leaf axil, of each plant in the four-plant sampling unit. This composite onion tissue sample weighed 1.5 g. Detection of IYSV was carried out using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA; Agdia, Inc., Elkhart, IN), following the procedure outlined in Voller et al. (1976) and using healthy negative and synthetic positive controls provided by the manufacturer (Agdia, Inc.,). Absorbance or optical density at 405 nm was measured using a BioTek ELx 800 plate reader (BioTek, Winooski, VT). Samples from each sampling unit were duplicated in microtiter plates, with the mean optical density for each unit determining the sample absorbance. A sample was considered IYSV positive if the mean absorbance values exceeded twice the negative control (User Guide: 2021 DAS-ELISA Reagent Set, Agdia Inc.).

Statistical analyses. Data from the *Proximity of seeded onion fields to transplanted onion fields on IYSV risk study* were analyzed separately each year due to variations in weather, growing conditions, IYSV incidence, and the number of sampling events. IYSV incidences were compared using repeated measures analysis of variance (ANOVA), followed by pairwise comparisons between field types, as well as two-sample *t*-tests ($P < 0.05$). Effect size was quantified as (η2g), representing the variance explained by each term in the model. An (η2g) value of 0.01 indicated a small effect, while values of 0.06 and 0.14 indicated medium and large effects, respectively. Data were analyzed using R statistical software R 4.0.3 (R core team 2020) and the following packages: 'epiphy' (Gigot 2018), 'rstatix,' and 'ggpubr' (Kassambara et al. 2020).

Data in the *Effect of onion plant size on IYSV risk study* were analyzed using Welch's two sample *t*-test of mean IYSV incidence in onion fields containing either large or small plants ($P < 0.05$). Samples were calculated using the mean IYSV incidence values (positive plant sample (+) per sampling quadrat) ($P < 0.05$). Data were analyzed using R statistical software R 4.0.3 (R core team 2020) and the following packages: 'epiphy' (Gigot 2018), 'rstatix,' and 'ggpubr' (Kassambara et al. 2020).

Results

IYSV incidence in seeded fields in proximity to transplanted fields. The seasonal mean incidence of IYSV-infected onions was three times greater in 2021 (46% incidence over four sample dates; Fig. 1A) than in 2022 (17% incidence over five sample dates; Fig. 1B). Overall, the mean incidence of IYSV did not significantly differ in fields established from transplants, seeded fields bordering transplanted fields or isolated seeded fields on any sampling date in 2021 or 2022 ($P > 0.05$; Fig. 1A and 1B). Similar incidences of IYSV observed among all field types in 2021 and 2022 suggested that proximity to transplanted fields did not increase the IYSV risk to onion fields.

Thrips density in seeded fields in proximity to transplanted fields. Seasonal mean populations of thrips per plant in 2021 (16 thrips per plant; Fig. 2A) was over twice as high as those in 2022 (7 thrips per plant; Fig. 2B). Mean densities of onion thrips did not significantly differ among those from transplanted fields, seeded fields bordering transplanted fields or isolated seeded fields on any sampling date in 2021 or 2022 ($P > 0.05$; Fig. 2A and 2B). Similar thrips densities observed among all field types in 2021 and 2022 suggested that proximity to transplanted fields did not increase thrips populations.

IYSV incidence in onion fields containing either large or small plants. The mean IYSV incidence in onion fields containing large plants was significantly greater (70% incidence) than those in onion fields containing small plants (16% incidence) on 24 July 2023 ($P = 0.01$; Fig. 3), this represents a 4.4-fold increase in IYSV incidence between onion fields comprising of either large or small plants during mid-season. IYSV incidence ranged between 40 and 92% in fields with large plants, whereas the range was 8 to 36% in fields with small plants.

The mean height of onion plants in fields with large plants was significantly greater than in fields with small plants ($P < 0.0001$), with the mean onion leaf height being 42.8% greater in fields with large plants than in those with small ones (Fig. 4A). The mean onion neck width of onion plants in fields containing large plants was significantly greater than those containing small plants ($P < 0.0001$), with the mean neck width being 38.5% greater in fields with large plants than in fields with small plants (Fig. 4B). The mean number of onion leaves per onion plant in fields with large plants was significantly greater than those in fields with small plants ($P < 0.0001$), with the mean number of onion leaves per plant being 52.5% greater in fields with large plants than in fields with small plants (Fig. 4C).

Discussion

IYSV incidence and onion thrips densities were similar among transplanted fields, seeded fields bordering transplanted fields, and isolated seeded fields on all sampling dates in 2021 and 2022. Contrary to our hypothesis, results indicated that the proximity of seeded onion fields to transplanted fields had no impact on IYSV incidence or onion thrips densities in seeded onion fields. These results expand on those by Komondy et al. (2023) who reported similar IYSV incidence and onion thrips densities between transplanted and seeded onion fields. Our findings contrast previous results that suggested transplanted onion fields were the major source of IYSV epidemics early to mid-season in New York onion fields. Leach et al. (2018) reported that the estimated number of viruliferous onion thrips in transplanted onion fields mid-season was 7 to 20 times higher than those in seeded onion fields. The reason why we did not detect differences in IYSV incidence and thrips densities among transplanted and seeded fields in our study may be explained by the variation in onion plant sizes. Transplanted fields were established from early April to late May, while seeded fields were planted from late March to mid-April. Sizes of plants in some transplanted and seeded fields may have been similar during thrips colonization, which could have explained the lack of differences in IYSV and thrips densities between the two field types. An increase in acreage devoted to transplanted onions might also explain the increase in frequency and severity of IYSV epidemics in the Elba Muck.

Because plant size may be implicated in thrips host-preference, we hypothesized that the overwhelming abundance of IYSV incidence would be found in onion fields containing large plants compared with those containing small plants. Our results indicated that seeded onion fields containing large plants are at greater risk for a higher incidence of IYSV than seeded onion fields containing small plants during mid-season. The mean incidence of IYSV in onion fields with large plants was four times greater (70% incidence) than those with small plants (16% incidence). Plant height, neck width, and leaf number per plant from fields containing large onion plants were significantly greater than those in fields containing small plants. These findings suggest that large onion plants may be more attractive and suitable for adult onion thrips colonization, and may likely explain the higher IYSV incidences in these fields during mid-season. Consequently, onion fields containing small plants will be at lower risk for IYSV infection during the thrips colonization period, thereby mitigating risk for reductions in bulb size and quality as well as the probability that the crop will be killed prematurely.

Other studies have demonstrated that planting time can affect virus incidence and insect pest density in agricultural cropping systems. McKirdy and Jones (1997) found that sowing wheat earlier in the planting season increased the overall incidence of barley yellow dwarf virus (BYDV) and its insect vector, the bird cherry-oat aphid, *Rhopalosiphum padi*. Results indicated that throughout the growing season, the earliest sown wheat fields had an average BYDV incidence three times greater than the latest sown wheat fields (McKirdy and Jones 1997). Similarly, Broadbent et al. (1952) found that planting 'Majestic' potatoes later in the season resulted in decreased incidences of the aphid-transmitted potato leafroll virus and potato virus Y.

Our findings have significant implications for the management of IYSV in the Elba Muck onion production region. Results from this study indicate that growers may no longer need to preferentially focus their onion thrips and IYSV control efforts on transplanted onion fields early in the season. Rather, onion fields (regardless of how they are planted) containing the largest plants early to mid-season during onion thrips colonization should be considered a priority for thrips management to help reduce the risk of initiating IYSV epidemics.

In the western U.S., spatially isolating onion fields is a tactic that has been implemented to curtail the spread of IYSV from seed production fields to nearby bulb production fields (Gent et al. 2006). In the Elba Muck region, multiple onion growers transplant and seed onions in the same general area and spatially isolating fields that might be predicted to contain either large or small plants during mid-season would be logistically difficult.

Our findings underscore the need for integrated pest management strategies that take into account plant size, regardless of plant type or planting time, at the time onion thrips colonize onion fields. Such strategies might involve extra early-season insecticide applications in fields that would be expected to contain the largest plants in the region. This will require thorough monitoring of thrips populations and IYSV incidence. Furthermore, our study highlights the importance of ongoing research and surveillance to better understand the dynamics of IYSV spread and thrips populations in this region, enabling growers to make informed decisions for the sustainable management of these significant threats to onion production.

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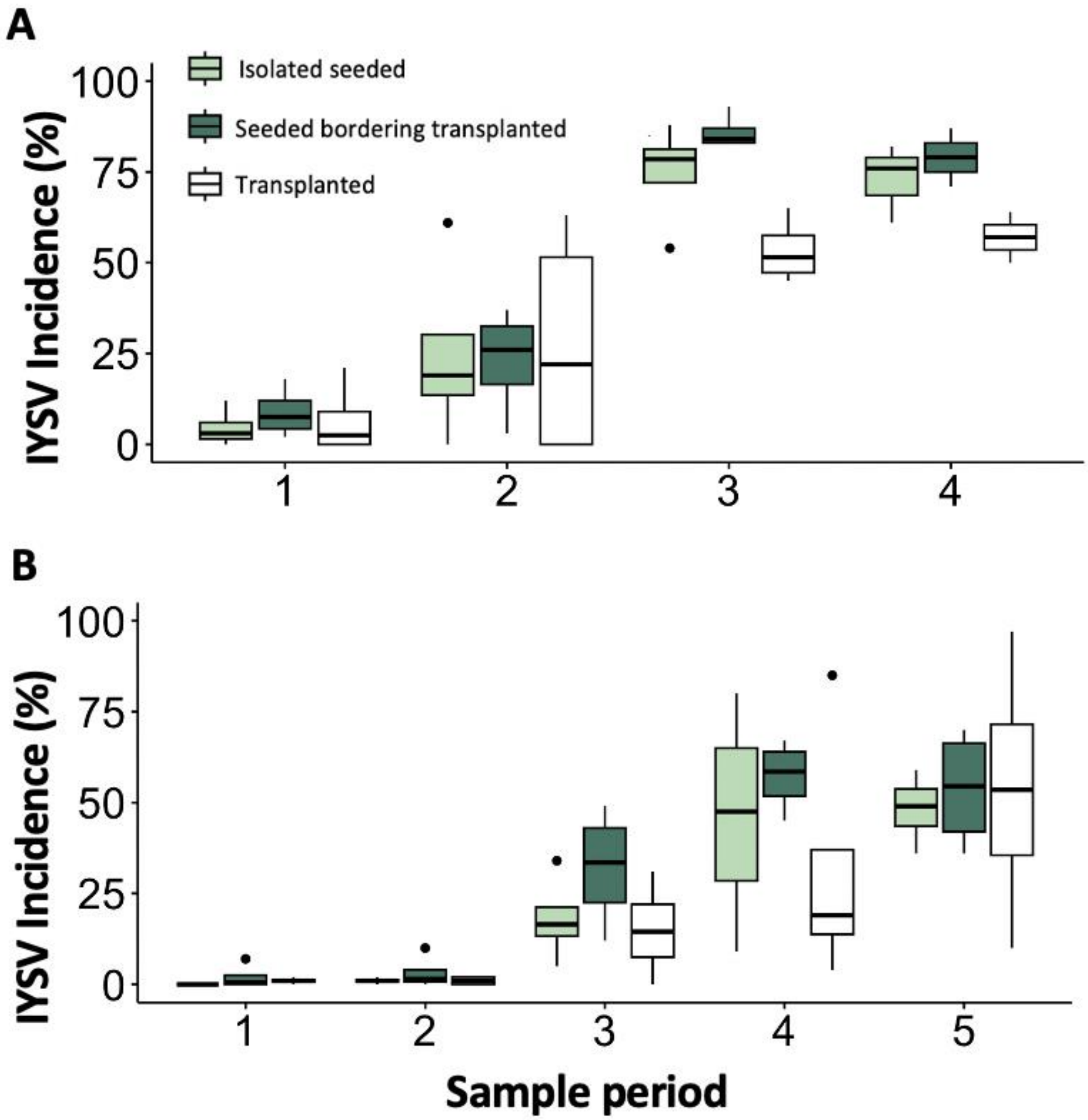

Fig. 1. Repeated measures analysis of mean incidence of iris yellow spot virus (IYSV) incidence in transplanted fields, seeded fields bordering a transplanted field, and isolated seeded onion fields during the 2021 (A) and 2022 (B) seasons in New York. Samples were calculated using the mean IYSV incidence values (positive plant sample (+) per sampling quadrat) in 2021 and 2022 ($P < 0.05$). Sample periods 1 through 4 in 2021 correspond with sampling dates beginning 14 and 28 June, 12 July, and 2 August. Sample periods 1 to 5 in 2022 correspond with sampling

dates beginning 27 June, 11 July, 1, 8 and 15 August. Mean incidence of IYSV per sampling unit among treatments was compared in each year. Fig. 1A: Repeated measures Anova, $F(2,38) = 1.1$, $p = 0.34$, $\eta^2_g = 0.06$; Fig. 1B: Repeated measures Anova, $F(2,54) = 0.16$, $p = 0.85$, $\eta^2_g = 0.006$. Note: some data points are not contained within box plots.

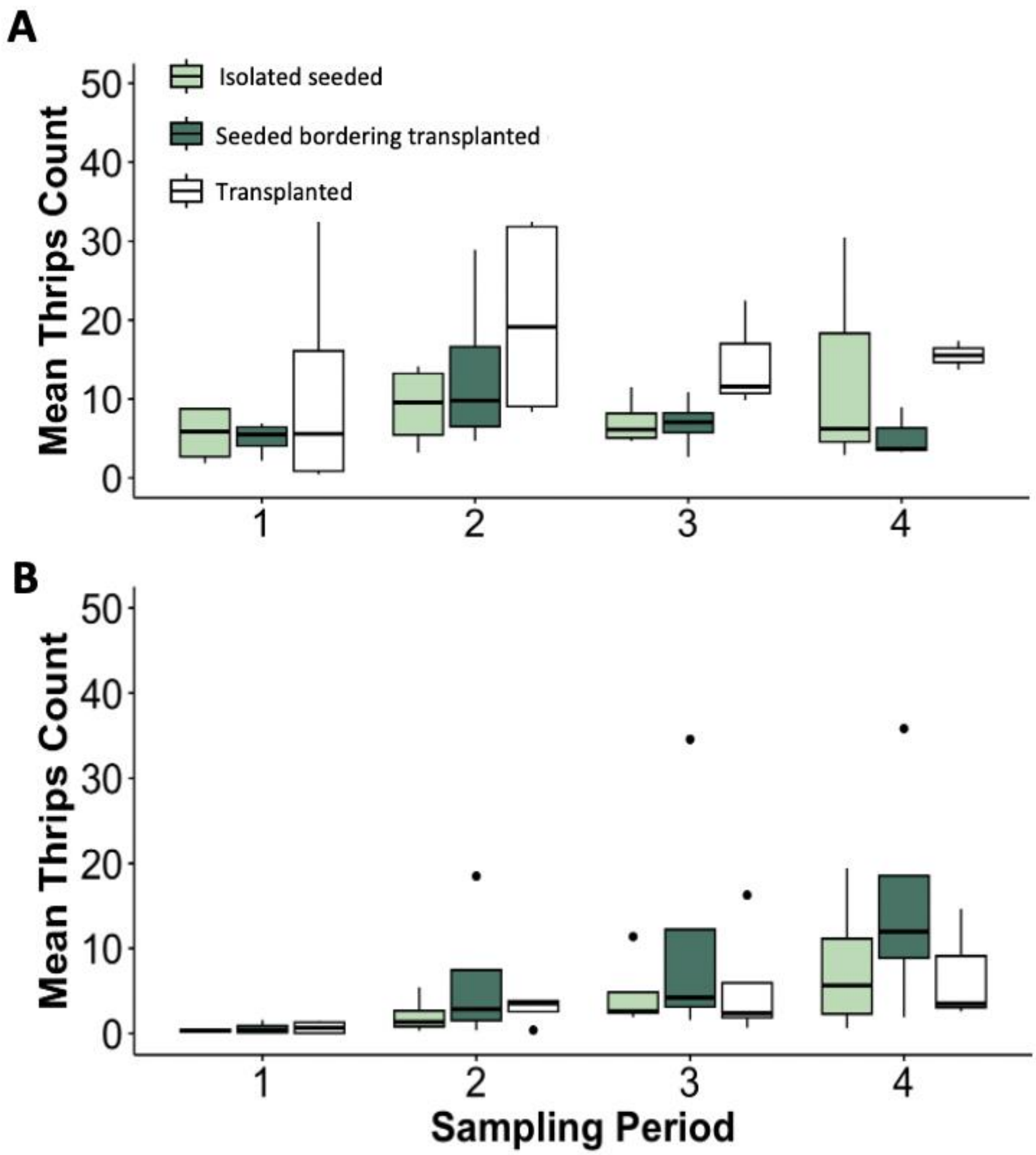

Fig. 2. Repeated measures analysis of mean number of onion thrips per leaf in transplanted, seeded fields bordering a transplanted fields, and isolated seeded onion fields in New York. Samples were calculated using the mean number of onion thrips found on each plant (total thrips density per plant, per sampling quadrat) in 2021 (A) and 2022 (B). Sample periods 1 through 4 in 2021 correspond with sampling dates beginning 14 June, 28 June, 12 July, and 2 August. Sample periods 1 through 5 in 2022 correspond with sampling dates beginning 27 June, 11 July, 1

August, 8 August, and 15 August. Mean number of thrips per sampling unit were compared in each sample period. Fig. 2A: Repeated measures Anova, $F(2,38) = 0.49$, $p = 0.61$, $\eta^2_g = 0.03$; Fig 2B: Repeated measures Anova, $F(2,42) = 0.7$, $p = 0.5$, $\eta^2_g = 0.03$. Note: some data points are not contained within box plots.

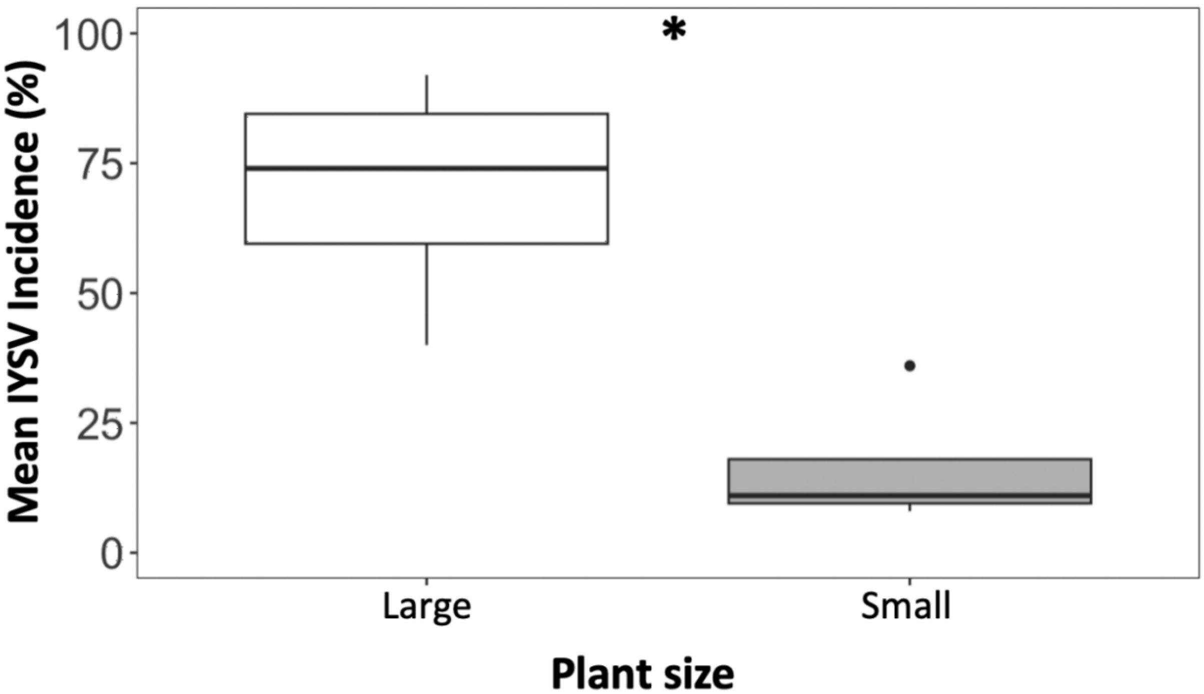

Fig. 3. Welch's two sample *t*-test of mean iris yellow spot virus (IYSV) incidence in onion fields containing either large or small plants on 24 July 2023 in New York. Samples were calculated using the mean IYSV incidence values (positive plant sample (+) per sampling quadrat) ($P < 0.05$). IYSV incidence was greater in fields with large plants than in those with small ones ($t(4.81) = 4.08$, $P = 0.010$). Note: some data points are not contained within box plots; asterisk indicates significance.

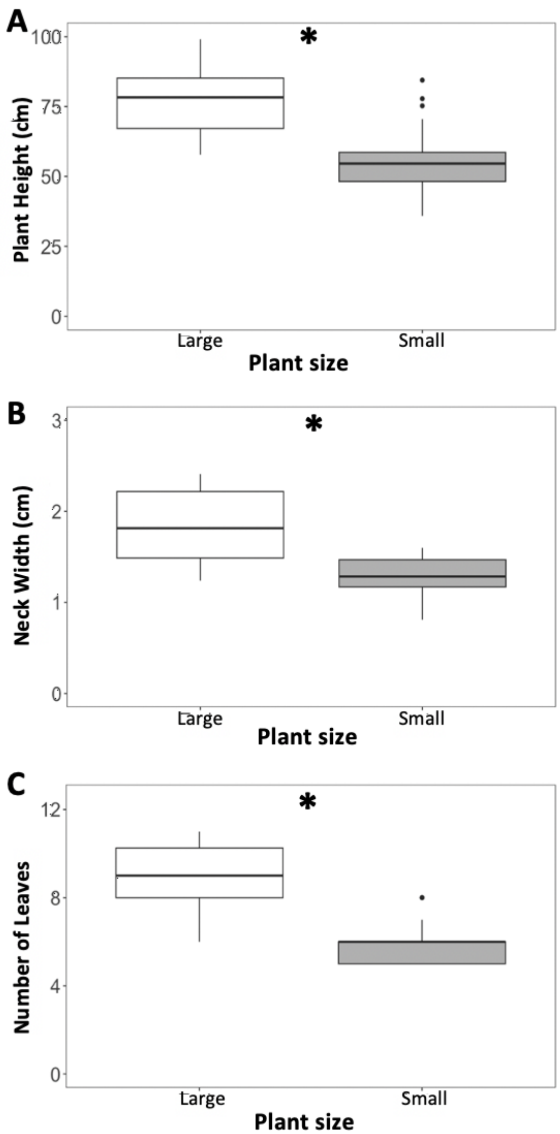

Fig. 4. Welch's two sample *t*-tests of mean plant height, onion neck width, and number of onion leaves per plant, per field in fields containing either large or small plants on 24 July 2023 in New York. Mean plant height ($t(37.97) = 6.25$, $P < 0.0001$), neck width ($t(27.22) = 6.01$, $P < 0.0001$) and number of leaves ($t(29.67) = 7.28$, $P < 0.0001$) were greater in fields containing large plants than those with small ones. Note: some data points are not contained within box plots; asterisk indicates significance.

Supplementary Table S1. Information on onion fields sampled in the Elba production region of New York in 2021 and 2022. Seeded onion fields were either bordering or isolated from transplanted onion fields.

2021	Field number	Field code	Field type	Onion variety
	Days to Maturity	Planting date		
	1	T	Transplant	Oneida
			105	
		24 Apr		
	2	S-T	Seed bordering transplant	Montclair
	112		07 Apr	
	3	I-S	Isolated seed	Montclair
		112		07 Apr
	4	T	Transplant	SV4643NT
	110		09 Apr	
	5	S-T	Seed bordering transplant	Safrane
	105		19 Apr	
	6	I-S	Isolated seed	Safrane
	105		19 Apr	
	7	T	Transplant	Hamilton
	120		25 May	
	8	S-T	Seed bordering transplant	Hamilton
	120		08 Apr	

	9	I-S	Isolated seed	Hamilton
	120		08 Apr	
	10	T	Transplant	Bradley
	118		27 May	
	11	S-T	Seed bordering transplant	Bradley
	118		23 Mar	
	12	I-S	Isolated seed	Hamilton
	120		25 Mar	
2022				
	1	T	Transplant	Hamilton
	120		29 May	
	2	S-T	Seed bordering transplant	Crockett
	114		14 Apr	
	3	I-S	Isolated seed	Crockett
	114		14 Apr	
	4	T	Transplant	Saffrane
	105		03 May	
	5	S-T	Seed bordering transplant	Safrane
	105		16 Apr	
	6	I-S	Isolated seed	Safrane
	105		16 Apr	
	7	T	Transplant	Cartier
	103		30 Apr	

8	S-T	Seed bordering transplant	Safrane
105		22 Apr	
9	I-S	Isolated seed	Safrane
105		22 Apr	
10	T	Transplant	Bradley
118		29 May	
11	S-T	Seed bordering transplant	Hamilton
120		06 Apr	
12	I-S	Isolated seed	Hamilton
120		06 Apr	

Supplementary Table S2. Plant size characteristics for onion plants sampled from seeded onion fields containing either large or small plants on 24 July (mid-season). This study was conducted in the Elba onion production region in 2023.

Field no.	Plant Size	Onion variety	Planting date	Plant height (cm)	Neck width (cm)	No. leaves
1	Large	Crockett	27 Apr	74.04 ± 4.47	1.59 ± 0.07	7.80 ± 0.37
2	Large	Crockett	27 Apr	74.30 ± 9.85	1.60 ± 0.23	8.50 ± 1.04
3	Large	Saffrane	01 May	81.35 ± 3.89	2.08 ± 0.10	9.40 ± 0.40
4	Large	Crockett	01 May	85.42 ± 6.14	1.99 ± 0.15	10.00 ± 0.82
5	Small	Saffrane	22 May	45.68 ± 2.46	1.24 ± 0.09	5.71 ± 0.29
6	Small	Saffrane	22 May	58.04 ± 1.61	1.23 ± 0.07	5.43 ± 0.20
7	Small	Saffrane	22 May	49.43 ± 2.02	1.32 ± 0.09	5.29 ± 0.18
8	Small	Hamilton	22 May	74.11 ± 3.66	1.37 ± 0.06	7.6 ± 0.24