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Epidemiology and Economic Impact of Impatiens Necrotic Spot Virus: A Resurging Pathogen Affecting Lettuce in the Salinas Valley of California

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Abstract

The Orthotospovirus impatiens necrotic spot virus (INSV) is a thrips-transmitted pathogen of lettuce that has rapidly emerged as a serious threat to production in the Salinas Valley of Monterey County, California. As a first step toward understanding the severity of the virus, we utilized Spatial Analysis by Distance IndicEs (SADIE) to characterize the distribution and progression of INSV outbreaks and thrips infestations in two commercial lettuce fields. In both fields, INSV incidence rapidly increased from 15.86% ± 1.77 to 80.24% ± 2.60 over the course of 7 weeks and aggregated at specific edges in both fields as early as 3 weeks after planting ($I_a = 1.63$, $P_a = 0.0100$, and $I_a = 1.53$, $P_a = 0.0300$). In one of the fields, thrips populations aggregated in areas that also experienced the most INSV ($I_a = 1.2435$, $P_a = 0.0400$, week 3; $I_a = 1.4815$, $P_a < 0.0001$, week 6; $I_a = 1.5608$, $P_a < 0.0001$, week 9), while in the second field, thrips were distributed randomly despite the aggregated effects that were observed for INSV incidence. Economic analysis estimated that the virus accounted for over $475,000 in losses for the two fields, while stakeholder surveys documented over 750 fields that experienced INSV infection during the 2021 season in Monterey County alone. These studies enhance our knowledge on the epidemiology of thrips and INSV under current lettuce production practices in the Salinas Valley, while elucidating the economic consequences and broader challenges that are associated with managing thrips-transmitted viruses.

Keywords: disease control and pest management, epidemiology, *Frankliniella occidentalis*, INSV, lettuce, numeric severity scale, thrips, virology

California leads the United States in the production of lettuce (*Lactuca sativa*), a high-value leafy vegetable (California Department of Food and Agriculture 2021). Over 60% of the state's production occurs in Monterey County, which is situated on the Central Coast of California and is home to the Salinas Valley. Monterey County lettuce is valued at over $1 billion annually, with hundreds of varieties grown across ~100,000 ac to supply domestic and international markets, including whole head lettuce or hearts, babyleaf lettuce, spring mix lettuce, or processing for pre-mixed salads (California Department of Food and Agriculture 2021; Monterey County Agricultural Commissioner's Office 2021). Lettuce types vary and include romaine, head, leaf, and specialty types, all of which can influence in-field production operations that are dependent on a variety's time to maturity, time of season at which it is grown, and the market it is intended for. The mild climate in Monterey County permits a long lettuce growing season, with plantings beginning as early as January and final harvests occurring in November. Due to these conditions, along with a relatively quick time to maturity (~70 to 100 days for romaine and head lettuce), a grower can typically plant multiple crops of lettuce on a per acre basis every season. Together, this paints a complex and intense production system for lettuce within the agriculturally diverse region of the Salinas Valley and Monterey County, California.

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Impatiens necrotic spot virus (INSV; family *Tospoviridae*, genus *Orthotospovirus*) is a thrips-transmitted pathogen that was first documented in lettuce in Monterey County in 2006 (Koike et al. 2008). Field surveys conducted in 2008 and 2009 documented INSV in 10 commercial lettuce fields at incidences ranging between 0.5 and 27%, with an average incidence of 5.7% per field (Kuo et al. 2014). Symptoms of INSV infection of lettuce include leaves that exhibit tan to dark brown necrotic spots, distorted leaf shapes, and stunted plant growth (Koike et al. 2008; Kuo et al. 2014) (Fig. 1). In some cases, brown necrotic lesions can be restricted to the inner midribs of the plant but is less commonly observed than those typically observed on leaves (Fig. 1). All lettuce types, including the two most grown lettuce types in Monterey County, romaine and heading types, are susceptible to INSV. A second orthotospovirus, Tomato spotted wilt virus (TSWV), also infects lettuce and causes similar symptoms, but its occurrence is much less frequent than INSV within Monterey County (Kuo et al. 2014). While the availability of lettuce cultivars with resistance to INSV is lacking, breeding efforts are ongoing (Simko et al. 2018).

Viruses within the *Orthotospovirus* genus (family *Tospoviridae*), including INSV and TSWV, infect hundreds of host plants, many of which are economically important food and ornamental crops globally (Pappu et al. 2009). All orthotospoviruses have enveloped virions that encapsidate a single-stranded RNA genome that is structured into three segments, large (L), medium (M), and small (S) and encode six proteins. The L protein is an RNA-dependent RNA polymerase that replicates and transcribes the viral genome; the nucleocapsid protein (N) serves to encapsidate the RNA genome; the NSs protein is an RNA-silencing suppressor in plant and insect cells and is a movement protein in plant hosts and insect cells; and Gn/Gc proteins are glycoproteins, which stud the surface of the virion and may be involved in interactions with the thrips vector (Oliver and Whitfield 2016; Rotenberg and Whitfield 2018).

INSV and other orthotospoviruses are transmitted by at least 14 species of thrips, a diverse and polyphagous group of insects within the order Thysanoptera which is comprised of over 7,000 species worldwide (Riley et al. 2011; Ullman et al. 2002; Whitfield et al. 2005). Western flower thrips, *Frankliniella occidentalis* (suborder Terebrantia, family Thripidae, subfamily Thripinae) is a globally distributed and notorious vector for orthotospoviruses, including

INSV and TSWV (Rotenberg et al. 2020). Thrips are particularly difficult to control due to their small size (1 to 2 mm), cryptic behavior, demonstrated resistance to numerous insecticide groups, and ability to infest and feed on an extensive host range of cultivated and non-cultivated plant species that support their growth and reproduction (Rotenberg et al. 2020). Surveys conducted in Monterey County in 2008 and 2009 determined that *F. occidentalis* is the dominant thrips species found in lettuce crops (Kuo et al. 2014). Several additional thrips vectors of INSV have been described and include *Frankliniella fusca* and *Frankliniella intonsa* (Rotenberg and Whitfield 2018), but neither these nor other species were identified from previous surveys in the Salinas Valley (Kuo et al. 2014).

INSV was first documented in the Salinas Valley in 2006, and virus incidence had continued to occur at mild to moderate levels until the past several years when severe outbreaks started to occur throughout Monterey County (Grower-Shipper Association, personal communication). Since then, INSV has impacted lettuce

Fig. 1. Lettuce production in the Salinas Valley affected by the re-emerging Orthotospovirus, impatiens necrotic spot virus (INSV). **A,** Typical commercial landscape of lettuce production, containing three fields of lettuce at different stages of growth: germinating field in the foreground; field that is midway through the lettuce crop cycle to the right; field that's being harvested in the background. **B,** Different stages of INSV symptoms in head lettuce: onset of symptoms (right) and slightly more advanced symptoms, indicated by extensive brown necrotic spots (middle). **C,** INSV symptoms in a Romaine lettuce plant at the two-true leaf stage. **D,** Advanced INSV symptoms in Romaine lettuce, indicated by extensive brown necrosis, twisting of leaves, and stunted growth of new leaves. **E,** Advanced necrotic lesions on leaves of Romaine lettuce caused by INSV infection. **F,** Advanced necrotic symptoms of INSV in the mid-ribs and veins of a lettuce cultivar grown for bagged processed salad mixes. **G,** Thrips infestation and feeding damage on a Romaine lettuce plant at the two-true leaf stage. **H,** Various stages of INSV symptoms in Romaine lettuce. **I,** Early occurrence of INSV infection in a field of Romaine lettuce, causing severe necrosis, stunting of growth, and yield losses.

production throughout the Salinas Valley and entire Central Coast region of CA, causing severe economic losses with increasing incidence and severity, with 100% crop losses in several fields in 2019, 2020, and 2021, amounting to millions of dollars lost annually (Grower-Shipper Association, personal communication). The occurrence of INSV in lettuce has also been documented in neighboring counties, San Benito and Santa Cruz County, and desert lettuce-producing regions of southern California and Arizona (Hasegawa et al. 2022). In 2020, a task force was assembled by the Grower-Shipper Association (GSA) of Central California to address the severe challenges in managing thrips and INSV, as well as a second pathogen, *Pythium uncinulatum*, that causes Pythium wilt of lettuce. The work described here focuses on research conducted during the 2019 season and industry surveys conducted by the task force during the 2021 season, which sought to better understand the epidemiology and occurrence of thrips and INSV in commercial lettuce fields.

Materials and Methods

Field sites

In 2019, two commercial romaine lettuce fields on a single ranch were chosen for this study. Fields were located on the central and eastern side of the Salinas Valley, between the cities of Chualar and Gonzales, CA. Field 1 was grown from early August to early-October and was 4 ha (~9.9 ac) in size. Beds were 1 m (3.33 ft.) in width, with two seeding lines of lettuce planted/bed. Field 2 was grown from mid-August to mid-October and was 2.1 ha (~5.2 ac) in size. Beds were 2 m (6.66 ft.) in width, with six seeding lines of lettuce planted/bed. The two fields were near one another, with 165 m (543 ft.) of separation between the east corner of Field 1 and the west corner of Field 2 (Fig. 2). Both fields were managed using conventional practices, and commercial operations were determined and conducted by the cooperating grower, including cultivar selection, seeding rate, irrigation scheduling, weeding, pesticide applications, and harvesting, and these practices were not influenced by this study. Both fields were sprinkler irrigated through germination, followed by drip irrigated after the thinning stage, which coincided with the beginning of the field evaluations (see below).

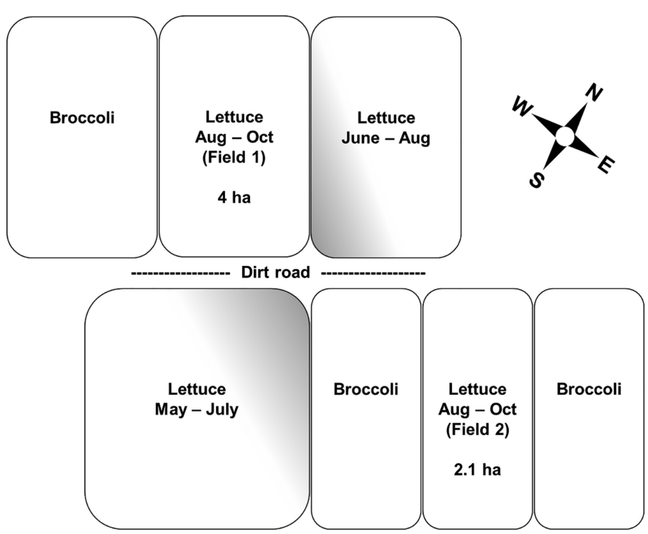

Fig. 2. Schematic of commercial lettuce fields used in the current study with respect to neighboring crops and their geographical orientation. The fields selected for the present study are referred to as Field 1 and 2, while adjacent lettuce fields with shaded gray indicate regions where impatiens necrotic spot virus (INSV) was confirmed prior to the studies conducted in Field 1 and Field 2. Months indicate the time when the lettuce crop was present in the system. The schematic represents a section of the entire ranch located between Chualar and Gonzales, CA, and was managed by a single grower using conventional practices.

In-field, non-destructive INSV evaluations

For both experimental fields, 25 equally distant scouting locations were marked in a 5 × 5 grid across the entire area of the field. At the center of each scouting location, 42 lettuce plants were selected, flagged, and used for weekly evaluations. In Field 1, the 42 plants were in a 3- × 2.1-m (9.99 × 7 ft.) area, which was comprised of three 1-m (3.33 ft.) beds wide (six lines of lettuce) × 2.1 m (7 ft.) in length. In Field 2, the 42 plants were in a 2- × 2.1-m (6.66 × 7 ft.) area and with only one bed that was 2 m (6.66 ft.) wide (six lines of lettuce) × 2.1 m (7 ft.) in length. The 42 plants in each of the 25 scouting locations were evaluated weekly for INSV symptoms, beginning after the thinning stage (week 3) and until the week prior to harvesting (week 9), for a total of seven sampling weeks at each field. Field 1 was harvested on October 4, 2019, and Field 2 was harvested on October 11, 2019. A numeric INSV severity scale ranging from 0 to 5 was used to evaluate the initial occurrence and progression of disease symptoms: 0 = no INSV symptoms; 1 = small and few brown necrotic spots with yellowing of leaves, sometimes accompanied by stunting of new growth in the heart; 2 = moderate levels of brown necrotic spots and yellowing of leaves, sometimes accompanied by stunting of new growth in the heart; 3 = high levels of brown necrotic spots and yellowing occurring in multiple leaves, stunting; 4 = obvious brown necrotic spots and yellowing occurring in multiple leaves including within the heart, stunting, distortion of leaves; and 5 = plant is dead (Fig. 3). To assess disease severity, the scores were averaged across the 42 plants at each of the 25 scouting locations every week. To assess disease incidence, the number of plants that were assigned an INSV score of 1 or greater were totaled across the 42 plants at each of the 25 scouting locations every week. The value was presented as a percentage of plants that exhibited INSV symptoms for each scouting location.

Confirmation of INSV infection

To confirm the presence of the virus, 10 lettuce plants exhibiting symptoms of INSV (severity scores ranging from 1 to 5; Fig. 3) were tested at week 3 using lateral flow serological tests (ImmunoStrips; Agdia, Elkhart, IN). The consistency and sensitivity of the method for detecting INSV was validated by comparing it to the triple antibody sandwich-enzyme-linked immunosorbent assay using monoclonal antiserum and manufacturer's protocols (Agdia, Elkhart, IN). All 10 plants, including those that were assigned an INSV severity score of 1, consistently tested positive for the virus using both methods, which validated the reliability of using the lateral flow serological tests to detect INSV, even at the onset of symptom development (data not shown). The diagnostic method was used throughout the remainder of the evaluation period as needed. For all samples, lateral flow serological tests were also used to simultaneously check for the presence of TSWV (ImmunoStrips; Agdia, Elkhart, IN). Over the course of the current study, TSWV was not detected in any of the tested samples (data not shown).

Progression over time of INSV symptoms in lettuce fields

Both disease severity scores and disease incidence were subjected to generalized mixed models (Proc Mixed; SAS Institute, Cary, NC), where the sole fixed factor was sampling week, with experimental field and replication nested with field, and the interaction between sampling week and replication were included as random factors. Sampling week was modeled as a repeated measure, where subjects were the combination of scouting locations and field. Degrees of freedom were calculated using the Kenward-Roger's procedure (Kenward and Roger 1997). Mean separation post analysis of variance was performed using the Tukey's test at $\alpha \leq 0.05$.

Thrips sampling from lettuce plants

To assess thrips abundance, a single randomly selected lettuce plant was collected south of the 25 evaluation locations every week in each field and brought back to the laboratory. The leaves from each plant were carefully removed, and adult thrips were collected into a

1.5-ml tube on ice using a paintbrush. The average number of adult thrips/plant and their SE was reported for each field.

Spatial distribution of INSV incidence and thrips infestations in lettuce fields

To characterize the patterns of INSV disease incidence in commercial lettuce fields, the Spatial Analysis by Distance IndicEs (SADIE) method was implemented. The SADIE method is a two-dimensional geostatistical approach that uses the relative locations of the sampling units, which in this case was the number of diseased plants per scouting location, to quantify the spatial arrangement of INSV-infected lettuce (Perry 1995; Perry et al. 1999). A randomization test was performed to calculate the distance to regularity (i.e., the state where each sampling unit of a given data set contains the same number of diseased plants) and provides data for the null hypothesis that the diseased plants were arranged randomly (Perry 1995; Perry et al. 1999). The index of aggregation, I_a, was reported, where $I_a > 1$ indicates aggregation of samples into clusters, $I_a = 1$ indicates a spatially random sample, and $I_a < 1$ indicates regularity. The test of the null hypothesis that the observed counts are arranged randomly was reported with a probability level, P_a. For each scouting location, the total number of plants that showed INSV symptoms was used for the SADIE procedure and computed using the epiphy package (v0.3.4) in R (R Foundation for Statistical Computing, Vienna, Austria). For each randomization test, 100 permutations were performed per scouting location per week to calculate cost flows using the Perry index. The values I_a and P_a are reported, and aggregation indices were mapped and presented as an interpolated landscape with contours.

Cost analysis of losses attributed to INSV infections

Two to 3 days after harvesting, a final in-field evaluation was conducted on the 42 plants at each scouting location. The number of plants that were not harvested was counted and used to estimate the economic losses associated with INSV infection. Only the plants that had clear symptoms of INSV were counted in the evaluation. While the price of lettuce can fluctuate greatly throughout the season, the price for romaine hearts is estimated to range between $9 and $21/carton, with each carton typically containing 12 three-count bags of romaine hearts. This range reflects the 2016 to 2018 3-year shipping point weekly averages of the USDA Agricultural Marketing Service for the Salinas region (Tourte et al. 2019). When Field 1 was harvested on October 4, 2019, romaine hearts were valued at $19.35 to $20.65/carton in the Salinas region. However, at the time Field 2 was harvested on October 11, 2019, the demand for romaine hearts had risen and was valued at $26.35 to $28.65/carton (USDA Agricultural Marketing Service). In the current study, a price of $20/carton was used in the analysis for Field 1, and $27/carton was used for Field 2. Typically, a grower can yield 2,472 cartons/ha (1,000 cartons/ac). However, for this study, conservative estimates were used in the analysis, at a rate of 2,224 cartons/ha (900 cartons/ac). Therefore, the gross return value was estimated at $44,480/ha ($18,000/ac) for Field 1 and $60,048/ha ($24,300/ac) for Field 2. The total costs, including operating costs (i.e., ground/bed preparation, irrigation, pesticide/herbicide applications, weeding, harvesting, etc.), land rent, taxes, and interest was estimated at $37,956/ha ($15,357/ac), with a return of 2,224 cartons/ha (900 cartons/ac; Tourte et al. 2019). The data is presented as number of cartons and dollars lost per hectare and acre for the two fields.

INSV industry self-reporting during the 2021 lettuce season

Beginning in 2019, numerous extension and educational events in the form of seminars, webinars, pest management meetings, and field visits were conducted with members of the lettuce industry to improve in-field scouting and recognition of INSV symptoms. Education and training events also included demonstrations on the use of rapid lateral flow serological tests for in-field diagnostics of INSV. During the 2021 lettuce season, a voluntary industry reporting system was launched by the task force and GSA of Central California to document the incidence of INSV throughout Monterey County. Members of the task force and reporting participants included 70 research scientists and extension personnel, growers, shippers, pest control advisors, certified crop advisors, members from the Monterey County Agricultural Commissioner's Office, and agriculture service companies working within Monterey County. Reports were submitted weekly to the GSA between the months of April and November. Only fields that had >1% INSV incidence were reported. Exact field locations and names of the participants were kept confidential, but in numerous cases, field visits were performed to confirm the presence of INSV. Plants were tested using ImmunoStrips to confirm infection, and visual observations were conducted to confirm the disease incidence that had been reported.

Results

In the summer of 2019, a grower reported a severe outbreak of INSV in two commercial romaine fields. In each field, plants exhibited symptoms of orthotospovirus infection, which included brown necrotic spots and stunted growth (Fig. 1). Ten symptomatic plants were collected from each field and tested for the presence of INSV and TSWV using lateral flow serological tests. All 10 samples from each field confirmed the presence of INSV and the absence of TSWV. The disease incidence was localized at the corners of the fields (Fig. 2). Several additional lettuce plantings were scheduled to take place in nearby blocks on the same ranch. Two of these newly planted fields were selected for INSV monitoring and are referred to as "Field 1" and "Field 2" throughout the remainder of this study. Both fields were directly seeded in August of 2019 with approximately 1 week separating the planting dates. The orientation of Field 1 and Field 2 are also shown in Fig. 2.

Fig. 3. Visual scoring system for documenting the severity of impatiens necrotic spot virus (INSV) symptoms in Romaine lettuce. Severity scores ranged from 0 to 5, where 0 = no INSV symptoms; 1 = small and few brown necrotic spots with yellowing of leaves, sometimes accompanied by stunting of new growth in the heart; 2 = moderate levels of brown necrotic spots and yellowing of leaves, sometimes accompanied by stunting of new growth in the heart; 3 = high levels of brown necrotic spots and yellowing occurring in multiple leaves, stunting of new growth; 4 = severe necrosis and brown spots evident in multiple leaves of the plant, stunting of new growth, distortion of leaves; and 5 = dead plant, often occurring only when infected at an early stage of growth.

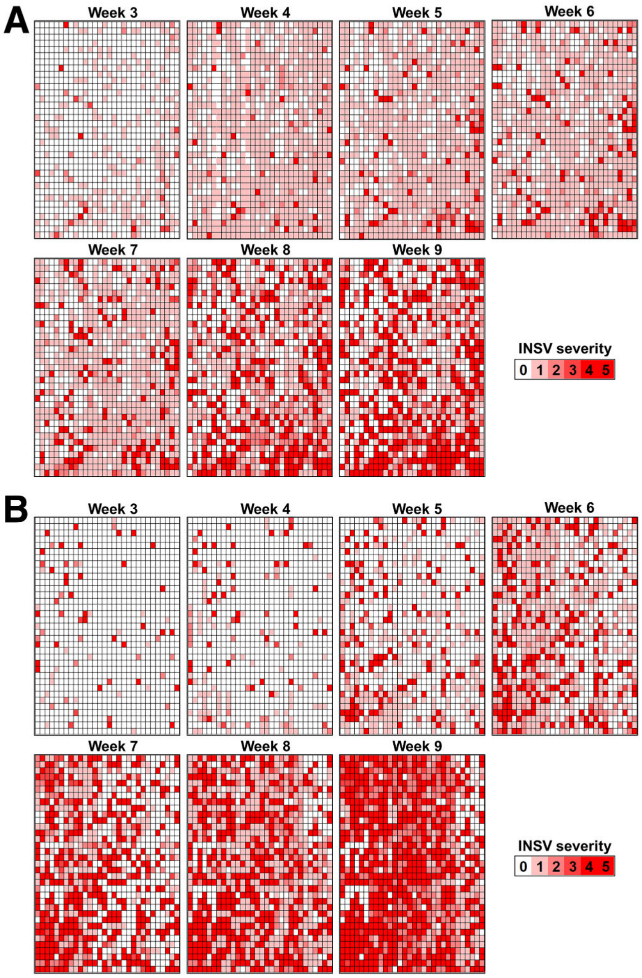

Fig. 4. Progression of impatiens necrotic spot virus (INSV) severity and incidence in two commercial fields. Individual lettuce plants were evaluated for INSV symptoms and assigned a severity score of 0 to 5, where darker red colors indicate greater severity. Each colored cell represents an individual plant and its INSV severity score. In-field, nondestructive evaluations were conducted on the same plants every week, beginning at week 3 (which followed the thinning stage of the lettuce crop) and ending at week 9 (which was prior to the scheduled field harvest). **A,** Field 1; **B,** Field 2.

Progression of INSV disease severity and incidence in commercial lettuce

In Field 1 and 2, symptoms of INSV infection were already present during the first evaluation period (week 3). Over the course of the next 7 weeks, field evaluations were conducted in both fields to document the progression of INSV severity and incidence. In total, 14,700 lettuce plants were evaluated and assigned an INSV severity score of 0 to 5 (Fig. 3). The scores for INSV disease severity at each of the 25 scouting locations significantly varied through the sampling weeks in both fields (df = 6, 54; F = 63.02; $P <$ 0.0001). On average, the lowest INSV scores were observed at week 3 and increased throughout the evaluation, with the highest average score (2.61 ± 0.12) being documented at the last evaluation, week 9 (Figs. 4 and 5, left panel). Additionally, INSV disease incidence in each scouting location drastically changed through time (df = 6, 56.3; F = 25.34; $P < 0.0001$). In both fields, the lowest incidence was documented at week 3 of the evaluation period, with 20% in Field 1, 3% in Field 2, and an average of 15.86% ± 1.77 in both fields. In contrast, the highest incidence was observed during the final evaluation period, week 9, where incidence was 67% in Field 1, 83% in Field 2, and had an average of 80.24% ± 2.60 between the two fields (Figs. 4 and 5, right panel). This data detailed the progression of INSV severity and incidence in two lettuce fields under commercial conditions.

Spatial distribution of INSV incidence and thrips infestations in commercial lettuce

Field 1 was adjacent to two older lettuce fields that were grown from May to July and June to August and experienced an estimated ~20 and ~30% incidence of INSV, with the highest incidence on the northeast corner and southwest corner of the fields, respectively (Fig. 4A). During the first evaluation period of Field 1 (week 3) INSV incidence was 20%. Further analysis on the spatial distribution of INSV revealed that disease incidence clustered in the southeast corner of the field ($I_a = 1.63$, $P_a = 0.0100$; Figs. 4A and 6A; Table 1). Subsequent analysis on the spatial distribution patterns of INSV each week demonstrated that INSV incidence consistently aggregated in the southeast corner of the field, including at the final evaluation period when incidence had reached 67% at week 9 ($I_a = 1.94$, $P_a <$ 0.0001; Figs. 4A and 6A; Table 1). Following the week 9 evaluation, a decision was made by the grower to not harvest the field, which equated to a 100% crop loss.

Field 2 was located to the southeast of Field 1, with a broccoli field planted between them and ~165 m of separation (Fig. 2). During the

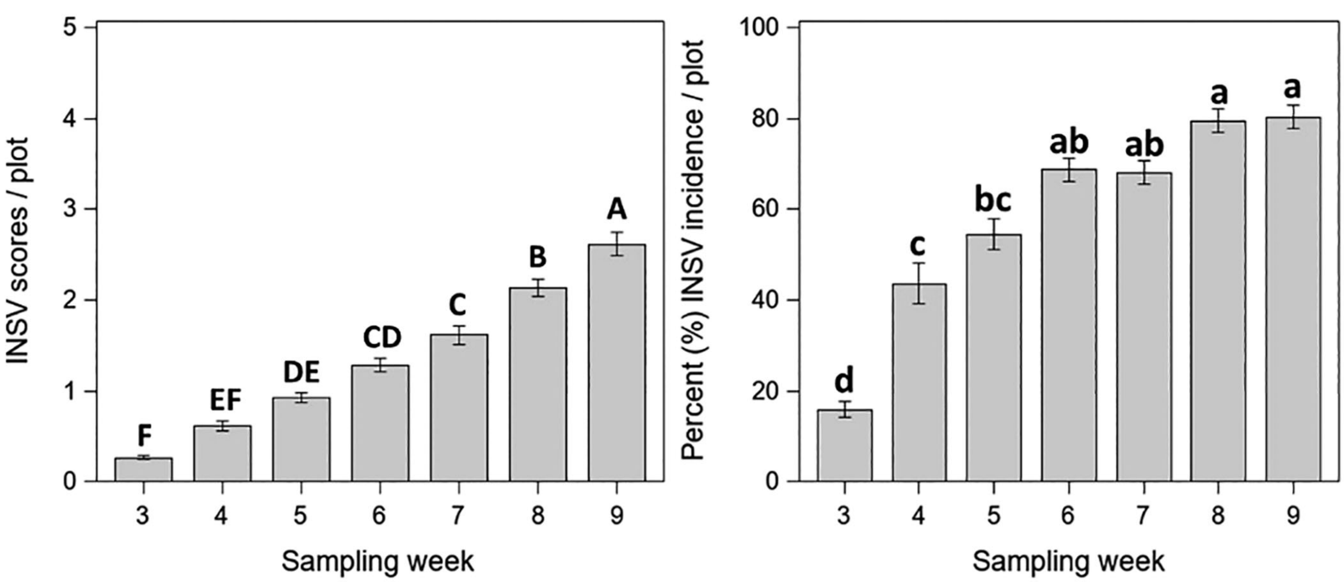

Fig. 5. Average impatiens necrotic spot virus (INSV) severity and incidence over time in commercial lettuce fields. Average INSV severity scores ± SE (left panel) and average INSV incidence ± SE (right panel) for all plants that were evaluated in both fields. Upper- and lower-case letters denote separate statistical analyses. Average bars with the same letters are not statistically different.

first evaluation of Field 2 (week 3) INSV incidence was 3%. Spatial analysis on the distribution of INSV demonstrated that disease incidence clustered on the west side of the field ($I_a = 1.53$, $P_a = 0.0300$). As INSV incidence and severity increased throughout the evaluation period, disease incidence also continued to aggregate on the west and southwest parts of the field at weeks 4, 5, 6, and 7 ($I_a = 1.63$ to 1.95, $P_a < 0.0001$; Figs. 4B and 6B; Table 1). However, INSV incidence did not aggregate at the final two evaluation periods, week 8 ($I_a = 1.10$, $P_a = 0.2000$) and week 9 ($I_a = 1.06$, $P_a = 0.2700$) (Figs. 4B and 6B; Table 1). This was likely due to the widespread incidence that

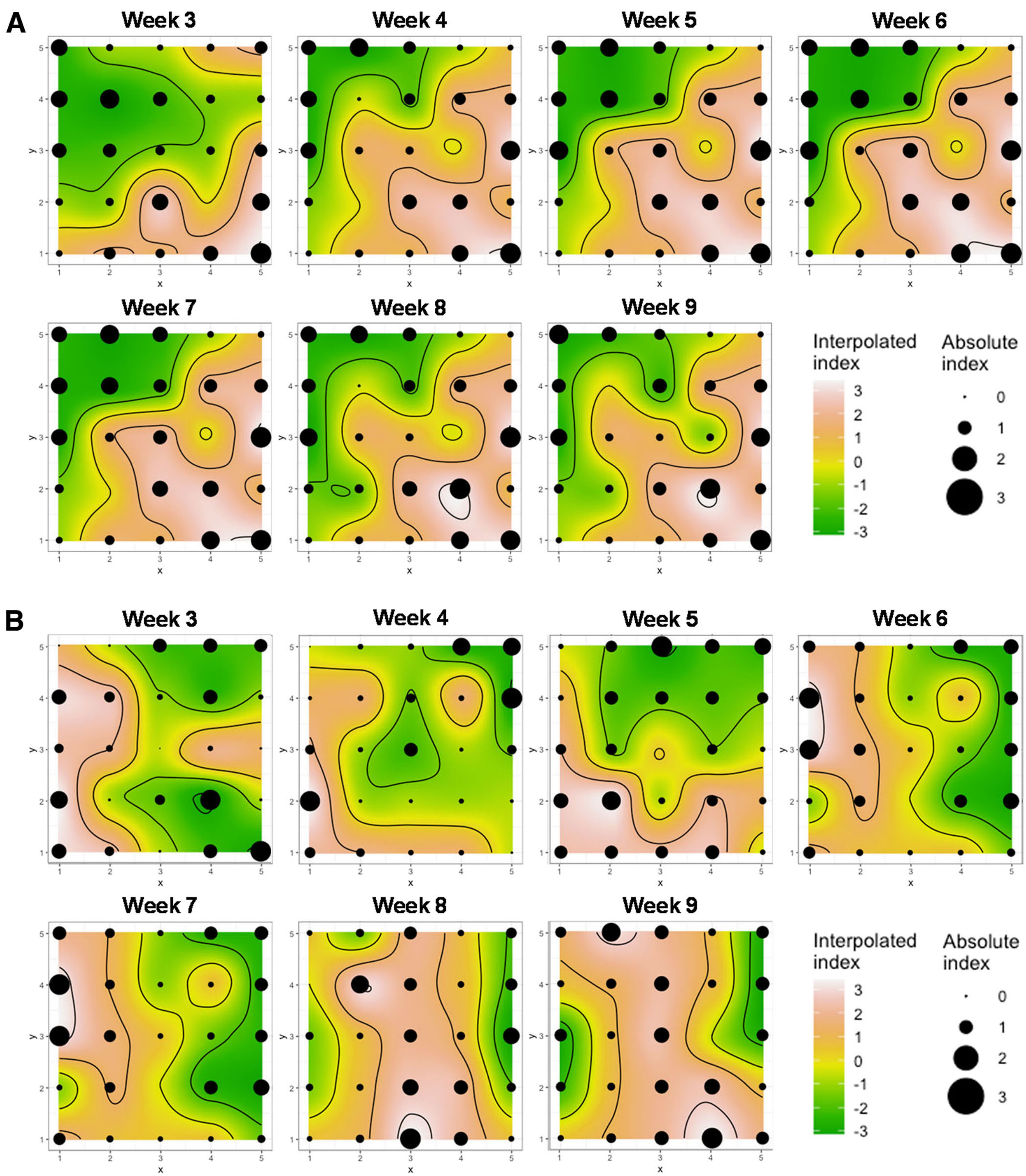

Fig. 6. Spatial analysis of impatiens necrotic spot virus (INSV) incidence in commercial lettuce fields. The total number of lettuce plants that received an INSV severity score greater than 0 was used to estimate disease incidence for each evaluation location across the two fields. Each evaluation location consisted of 42 plants that were evaluated weekly, with 25 locations arranged in a 5 × 5 grid in each field. Interpolation (red, yellow, and green colors) and absolute aggregation (black circles) indices were calculated using SADIE analysis. White and pink colors and larger circles indicate a higher likelihood where INSV incidence is aggregated, in comparison to yellow or green colors and smaller black circles. **A,** Field 1; **B,** Field 2.

had established throughout the field, where 83% of the plants exhibited minor to severe symptoms of INSV infection at week 9. Despite the high level of disease incidence, the field was harvested, and post-harvest evaluations determined that 26% of the plants were harvested, equating to a 74% crop loss. Further analysis revealed that on average, plants that received a final INSV severity score of 1.54 ± 0.05 during the week 9 evaluation still had market potential and were harvested as a result.

The methods that were used to assess the spatial patterns for INSV disease incidence were also used to analyze the in-field distribution of thrips. In Field 1, the number of adult thrips varied between 0 and 58 thrips/plant, with an average of 4.37 ± 0.74 thrips/plant. However, in contrast to the strong aggregated effects observed for INSV incidence in Field 1, thrips were distributed randomly for weeks 4 to 9 (I_a = 0.93 to 1.13, P_a = 0.15; Fig. 7A and Table 1). In Field 2, thrips populations ranged between two and 12 thrips/plant, with an average of 2.28 ± 0.42 thrips/plant. Thrips also aggregated in the southwest corner of Field 2 during week 3 (I_a = 1.24, P_a = 0.04), week 6 (I_a = 1.48, $P_a < 0.0001$), and week 9 (I_a = 1.56, $P_a < 0.0001$) (Fig. 7B; Table 1), which was similar to how INSV aggregated during weeks 3 to 7.

Estimations of economic impact caused by INSV infection

Field 1 experienced a 67% INSV incidence at week 9 but was considered a 100% crop loss due to the field not being harvested. With a return rate of $20/carton for romaine hearts and 2,224 cartons produced/ha (900 cartons/ac), the estimated loss in gross returns was estimated to be $178,200 for that field. Additionally, losses attributed to production costs were estimated to be $152,034, amounting to a total loss of $330,234 for the 4-ha field. Field 2 experienced 83% INSV incidence but was considered a 74% yield loss. With a return rate of $27/carton at the time Field 2 was harvested, gross return losses were estimated at $93,506, while $59,093 was spent on production costs, totaling $152,599 in losses for the 2.1-ha field. Between the two fields, total losses were estimated to be $482,833 for the one affected grower.

During the 2021 season, reporting of INSV between the months of March and November documented 766 commercial lettuce fields that were infected with INSV at an incidence higher than 1%. Impacted fields included all lettuce types, including romaine, head, leaf, butter, and specialty types, and both conventional and organic growers were affected. Further analysis of reports that were submitted by a single grower provided greater documentation of the severity of INSV during the 2021 season. The reports documented 23 fields across 89 ha (220 ac), with fields ranging in size between 1.2 and 6 ha/field (~3 to 15 ac/field) and consisted of 13 romaine lettuce fields, six head lettuce fields, and four leaf type lettuces. Fields were managed using conventional practices. INSV incidence ranged between 3 and 100%, with an average field incidence of 30% within infected fields for the grower during the 2021 season. Overall, it's estimated that ~25% of the entire lettuce industry and GSA members participated in the surveys, suggesting that INSV disease incidence and severity was underestimated, further highlighting the widespread impact that the virus has had on lettuce production in the Salinas Valley.

Discussion

Monterey County, which encompasses the Salinas Valley, is one of the most diverse and prolific agricultural regions in California. Lettuce occupies ~40,460 ha (~100,000 ac) annually but is only one of over 100 vegetable and fruit crops that are grown in the region. Many of these crops, including broccoli, cauliflower, brussels sprouts, celery, strawberries, cane berries, artichokes, spinach, and grapes are grown adjacent to lettuce or are rotated with lettuce crops (Monterey County Agricultural Commissioner's Office 2021). The dynamic production of a wide range of crops creates a complex agricultural system, which presents challenges in developing integrated pest management strategies for thrips and INSV, both of which have a broad host range of plant species that can support their growth.

Since its first report in 2006 (Koike et al. 2008), INSV has rapidly re-emerged in recent years, resulting in severe losses for the lettuce industry throughout the Salinas Valley. The current studies sought to document the severity and progression of INSV infection in commercial lettuce fields during the 2019 season. We specifically aimed to characterize the distribution of the virus in commercial fields that were managed using standard conventional practices by a single grower. Importantly, we wanted to achieve a level of resolution that would allow us to identify spatial patterns of disease incidence and thrips abundance using the SADIE method (Perry 1995; Perry et al. 1999) while providing the most accurate estimates on yield and economic losses that were associated with INSV infection. Working closely with various stakeholders, we also provided a summary of voluntary industry-wide surveys that documented the widespread occurrence of INSV throughout the Salinas Valley during the 2021 season.

Over the course of 7 weeks (week 3 to week 9 of the crop cycle), 1,050 lettuce plants in each of two commercial romaine fields were evaluated for INSV symptom severity and assigned a score between 0 and 5. In both fields, INSV severity and incidence increased over time until the fields were harvested. SADIE analysis highlighted edges of the experimental fields where the disease was concentrated, suggesting its proximity to an external source from which INSV originated. In Field 1, the southeast corner experienced the highest level of INSV, which was adjacent to the southwest corner of an older lettuce crop that was infected with the virus (Fig. 2). The data captured the early occurrence of INSV (20% disease incidence at week 3) in a newly planted lettuce crop that was adjacent to another crop where the virus was already present and later resulted in a disease incidence of 67% at week 9. However, in contrast to the concentrated disease incidence that occurred consistently each week until the harvest date, thrips were distributed randomly throughout the field. This suggests that while the adjacent lettuce crop served as a reservoir for INSV and viruliferous thrips populations, thrips infestations in other parts of the field occurred just as frequently.

Table 1. Aggregation values for impatiens necrotic spot virus (INSV) incidence and thrips abundance[a]

	Field 1				Field 2			
	INSV		Thrips		INSV		Thrips	
Week	I_a	P_a	I_a	P_a	I_a	P_a	I_a	P_a
3	1.6314	0.0100	N/A	N/A	1.5389	0.0300	1.2435	0.0400
4	1.8258	<0.0001	0.9594	0.5400	1.6330	<0.0001	0.9849	0.4600
5	1.8858	<0.0001	0.9384	0.6700	1.9517	<0.0001	0.9331	0.6300
6	1.8571	<0.0001	1.1366	0.1500	1.9034	<0.0001	1.4815	<0.0001
7	1.8732	<0.0001	0.9320	0.6300	1.8727	<0.0001	1.0259	0.3000
8	1.9031	<0.0001	1.0995	0.2600	1.1034	0.2000	1.1588	0.1000
9	1.9424	<0.0001	1.1274	0.1700	1.0652	0.2700	1.5608	<0.0001

[a] Aggregation values (I_a) and P values (P_a) from SADIE analysis for INSV incidence and thrips abundance from Field 1 and Field 2. Thrips were not recovered from lettuce during week 3 in Field 1, and thus, analysis was not performed for that week (indicated as N/A).

Spatial analysis conducted for Field 2 revealed a similar pattern where INSV was concentrated, specifically on the west and southwest edges of the field during weeks 3 to 7. However, during the last 2 weeks, INSV was distributed randomly in the field, resulting in a disease incidence of 83% at week 9. Thrips populations were also concentrated on the southwest edge during weeks 3, 6, and 9. In contrast to Field 1, the data supports the role for a common reservoir that resulted in a higher number of thrips and higher disease incidence in the southwest corner of the field. It remains unclear if the accumulation of thrips and INSV in Field 1 directly contributed to the high incidence of INSV observed in Field 2, considering the presence of a broccoli crop that separated the two fields (Fig. 2). Broccoli and other *Brassicas* are often rotated with lettuce, and it is common for a single ranch that is 50 to 200 ha (~123 to 494 ac) in size to have

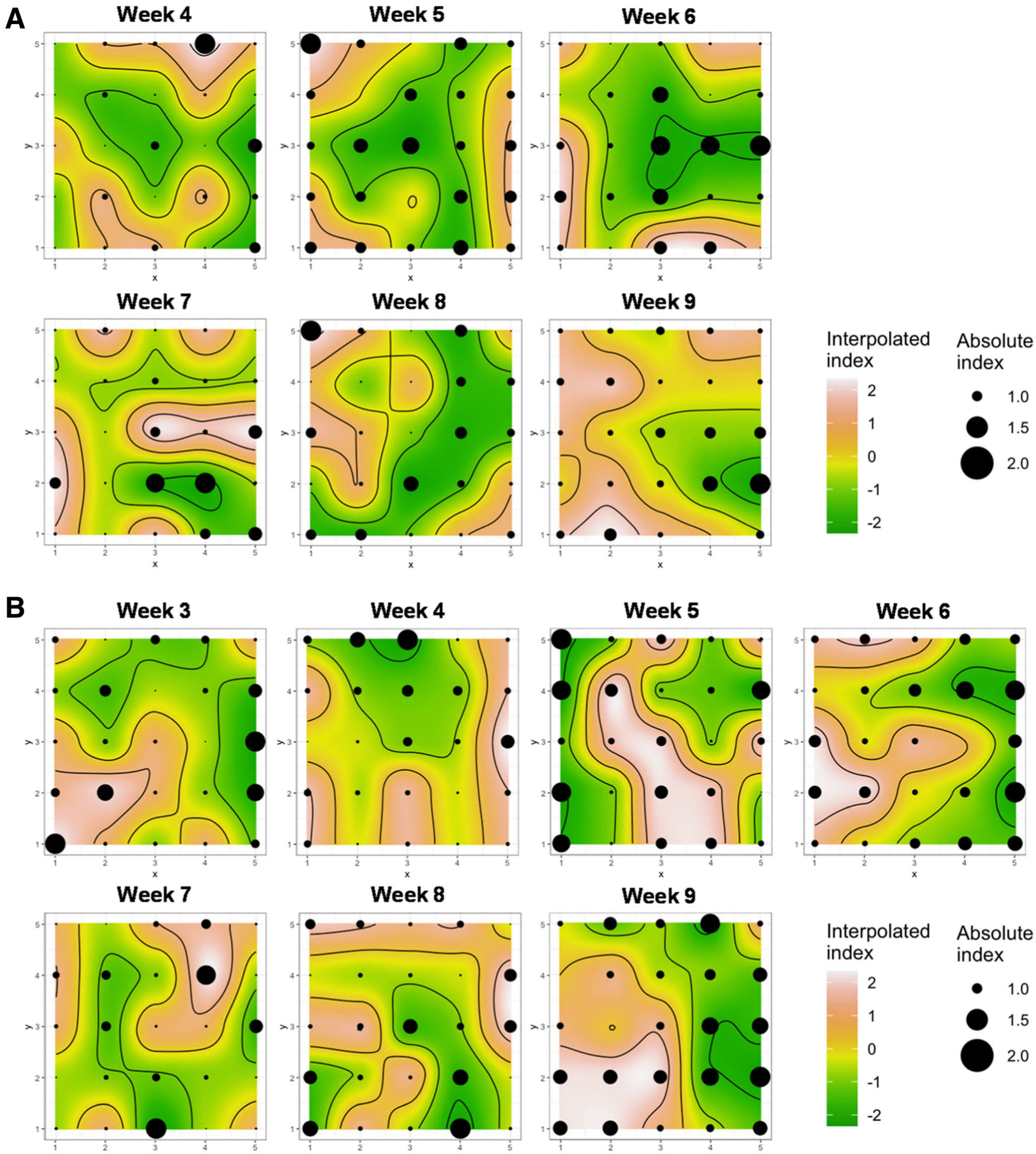

Fig. 7. Spatial analysis of thrips abundance in commercial lettuce fields. The number of adult thrips were counted from lettuce plants that were collected adjacent south of the 25 evaluation locations every week. Interpolation (red, yellow, and green colors) and absolute aggregation (black circles) indices were calculated using SADIE analysis. White and pink colors and larger circles indicate a higher likelihood of thrips abundance being aggregated, in comparison to yellow or green colors and smaller black circles. There was no interpolation and absolute indices calculated for week 3 in Field 1 since no thrips were recovered from lettuce plants. **A,** Field 1; **B,** Field 2.

various stages of lettuce and *Brassicas* growing in 3 to 6 ha (~7 to 14 ac) blocks, as illustrated in the present study (Fig. 1A). Past studies have shown that broccoli can support the growth of western flower thrips, but the crop is not considered a host for the virus (Joseph and Koike 2021; Kuo et al. 2014). Alternatively, it is possible that viruliferous thrips populations originated somewhere south of Field 2, but this area was outside the boundaries of the grower's ranch and thus was not accessible for further investigation at the time of this study. During the summer, strong winds are common in the Salinas Valley, often reaching speeds of 24 km/h, and likely plays an important role in dispersal of thrips vectors, as documented in other cropping systems (Fernandes and Fernandes 2015). It would be important to characterize the effect that wind has on thrips dispersal and INSV incidence.

This data captures the rapid spread of INSV in commercial lettuce crops on a single ranch that was managed using standard lettuce production and crop rotation strategies that are practiced throughout much of the Salinas Valley. The data underscores the notion that while thrips infestations can be detrimental by causing cosmetic feeding damage on marketable products, it is their role as virus vectors that poses severe risks to the production of a crop. The data also demonstrates the severe consequences that can occur when thrips vectors and INSV are introduced into the current commercial model for lettuce production, where lettuce is grown in small blocks on staggered planting schedules and occurs in high densities throughout a long growing season.

In the current study, the levels of INSV incidence and economic losses differed between the two fields. In Field 1, INSV incidence was 67% but was considered a 100% crop loss due to the grower's decision not to harvest the field and amounted to $330,234 in losses. In contrast, INSV incidence in Field 2 was 83% but was considered a 74% crop loss following a postharvest evaluation of the field, and economic losses were calculated to be $152,599. Interestingly, in Field 2, the number of plants that exhibited INSV symptoms was 9% higher than the number of plants that did not get harvested. This suggests that plants exhibiting only minor symptoms of INSV infection may still possess market value but would be dependent on the market the crop is grown for (e.g., romaine hearts, whole head lettuce, processed salad mixes, etc.). Furthermore, the discrepancy between INSV incidence and economic losses between the two fields largely reflects the complexity of the market and the numerous economic decisions that a grower must consider throughout the production of a crop. In the current study, Field 1 was scheduled to be harvested on October 4, 2019, when the market value for romaine hearts was $20/carton. Despite the higher incidence of INSV in Field 2, it was estimated that 26% of the crop was harvested on October 11, 2019, when the market value had risen to $27/carton. The fluctuating return rates for a carton of romaine hearts typically varies between $9 and $21/carton (Tourte et al. 2019), but during times of high demand, can reach upwards to $40/carton (Grower-Shipper Association, personal communication) and thus can strongly influence when and if a crop is harvested.

Surveys conducted during the 2021 growing season documented the widespread occurrence of INSV in over 750 fields, despite only ~25% of the industry reporting. While surveys did not occur during the 2020 season, separate surveys estimated that growing costs increased on an average of 10 to 15% per grower or approximately $1,483/ha (~$600/ac), with most of the costs being associated with an increase in pesticide use (Grower-Shipper Association, personal communication).

From an integrated pest management standpoint, several factors limit the implementation of strategies to manage thrips and INSV across the entire lettuce industry. In 2020, organic production of lettuce accounted for ~20% of the entire industry, while over 150 million tons of lettuce was produced for export markets, which can require different standards in quality and maximum residue limits for pesticides that are used on the crop (Monterey County Agricultural Commissioner's Office 2021). Currently, only limited chemical options exist for managing thrips in conventionally and organically produced lettuce within the state of California, and genetic-based resistance to INSV in lettuce cultivars is lacking. Therefore, research is needed to develop diverse and sustainable pest control tactics. Understanding the host range for INSV and identification of crops and habitats that support thrips abundance would be important for developing strategies to manage the vector and virus throughout the lettuce growing season that occurs between January and November, as well as during the months when lettuce is not grown. While the current study did not seek to identify the species composition of thrips that were collected from lettuce samples, previous studies in 2008 and 2009 found that 86 to 98% of the thrips species in lettuce from the Salinas Valley were western flower thrips, *F. occidentalis* (Joseph and Koike 2021; Kuo et al. 2014). Additional studies to recharacterize the diversity of thrips species in the Salinas Valley would be important.

The studies described here highlight the rapid re-emergence of INSV that has reached epidemic levels in lettuce produced in the Salinas Valley of California and throughout coastal production areas. Meanwhile, recognition of INSV as an important pathogen of both ornamental and vegetable crops continues to grow globally, as the virus has recently emerged in new geographic territories and hosts crops (Chung et al. 2021; Stanković et al. 2020; Zhao et al. 2018), which include other lettuce producing regions such as Arizona and the southern desert regions of California (Hasegawa et al. 2022) and Greece (Beris et al. 2020). Continuous efforts among researchers, academics, extensionists, and stakeholders will be critical for responding to the growing threat of INSV and thrips as virus vectors in the Salinas Valley of California and other regions.

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