Characterization and Epidemiology of Outbreaks of *Impatiens necrotic spot virus* on Lettuce in Coastal California

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Abstract

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California is the leading producer of lettuce (*Lactuca sativa*) for the United States and grows 77% of the country's supply. Prior to 2006, coastal California lettuce was only periodically and incidentally infected by a single tospoviruses species: *Tomato spotted wilt virus* (TSWV). However, beginning in 2006 and continuing through 2012, severe outbreaks of disease caused by *Impatiens necrotic spot virus* (INSV) have affected the coastal lettuce crop, though TSWV was also present. In contrast, TSWV was the only tospovirus associated with disease outbreaks in Central Valley lettuce during this period. Disease surveys conducted over two seasons (2008 and 2009) in 10 commercial fields (acreage of 6 to 20 ha) indicated that INSV was the only tospovirus associated with economically damaging disease outbreaks in lettuce in the coastal region, with incidences of 0.5 to 27% (mean = 5.7%). Molecular characterization of INSV isolates associated with these disease outbreaks revealed little genetic diversity and indicated that lettuce-infecting INSV isolates were nearly identical to those previously characterized from ornamental or other hosts from different locations in the United States and the world. Monitoring of thrips revealed moderate to large populations in all surveyed lettuce fields, and the majority of thrips identified from these fields were western flower thrips, *Frankliniella occidentalis*. There was significant positive correlation ($r^2 = 0.91$, $P = 0.003$) between thrips populations and INSV incidence in the most commonly encountered type of commercial lettuce (romaine, direct seeded, conventional) included in this study. A reverse-transcription polymerase chain reaction assay developed for detection of INSV in thrips showed promise as a monitoring tool in the field. Surveys for INSV reservoir hosts in the coastal production area revealed that the weeds little mallow (*Malva parvifolia*) and shepherd's purse (*Capsella bursa-pastoris*) were commonly infected. *M. parvifolia* plants infected in the field did not show obvious symptoms, whereas plants of this species inoculated in the laboratory with INSV by sap transmission developed necrotic spots and chlorosis. Eleven other weed species growing in the lettuce production areas were found to be hosts of INSV. Coastal crops found to be infected with INSV included basil (*Ocimum basilicum*), bell pepper (*Capsicum annuum*), calla lily (*Zantedeschia aethiopica*), faba bean (*Vicia faba*), radicchio (*Cichorium intybus*), and spinach (*Spinacia oleracea*). Thus, it is likely that INSV was introduced into coastal California lettuce fields via viruliferous thrips that initially acquired the virus from other local susceptible plant species. Results of this study provide a better understanding of INSV epidemiology in coastal California and may help growers devise appropriate disease management strategies.

Lettuce (*Lactuca sativa*) is a popular and high value leafy green vegetable in the family Asteraceae that is grown as a fresh-market commodity and is used extensively in salads. California is the leading producer of lettuce in the United States, with >83,450 ha grown in 2011 (44). This represented approximately 77% of the country's lettuce production (44). The value of lettuce in California in 2011 was $1.5 billion. In California, lettuce is mainly produced in seven contiguous coastal counties (from north to south: Santa Clara, San Benito, Santa Cruz, Monterey, San Luis Obispo, Santa Barbara, and Ventura), which annually account for 78% of the state's lettuce production (5). Monterey County, which is the location of the Salinas Valley, accounts for about 65% of California's lettuce (5). In this coastal region, lettuce is planted over a 9-month period, from January through September. Smaller acreages of lettuce are planted in the late fall to early winter and late winter to early spring months in California's San Joaquin Valley (Fresno, Kern, and Kings Counties) and southern desert regions (Imperial and Riverside Counties) (40,42). As a commodity, lettuce is a complex crop, with dozens of cultivars grown for each commercial type: iceberg (crisphead), romaine, greenleaf, redleaf, butterhead, and other specialty types (40,42). In addition, all of these types can be grown at different planting densities and for varying lengths of time depending on whether they will be harvested for babyleaf lettuce, teenage or spring mix lettuce, hearts (primarily romaine), whole-head lettuce, or value-added bagged lettuce products (in which the lettuce is harvested, chopped, washed, dried, and placed into controlled-atmosphere bags for ready-to-eat salads) (40,42).

Virus diseases are usually of minor concern for coastal California lettuce production (S. T. Koike, *personal observation*). Due to an integrated disease control program consisting of seed testing, weed management, a 1-month lettuce-free period, and crop residue plow-down regulations, the potyvirus *Lettuce mosaic virus* (LMV) has been managed effectively in this production region (12,52). *Mirafiori lettuce big-vein virus*, which causes lettuce big vein, is widespread but causes limited economic impact (S. T. Koike, *personal observation*). Two soilborne tombusviruses, *Lettuce necrotic stunt virus* and *Tomato bushy stunt virus*, are responsible for lettuce dieback disease, which causes occasional damage to romaine and leaf lettuces, particularly in areas that experience flooding (29). Other lettuce viruses occur sporadically, are found at low incidences, and are of little economic concern, including the luteovirus *Beet western yellows virus*, the tospovirus *Tomato spotted wilt virus* (TSWV), and the potyvirus *Turnip mosaic virus* (17). In the San Joaquin Valley, TSWV has been present in lettuce crops for many years but the prevalence and severity of outbreaks have increased dramatically in the August-planted crop (T. Turini, *personal observation*).

Beginning in 2006, severe outbreaks of a virus-like disease occurred in a number of commercial lettuce fields in the Salinas Val-

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ley. Symptoms of this disease included stunted plant growth, chlorosis, and leaves with irregular-shaped, tan to dark brown, necrotic spots or ringspots (Fig. 1). Older leaves showed extensive marginal necrosis and wilting. Lettuce plants infected at the seedling through rosette stage were severely stunted and did not form marketable heads (Fig. 1). These symptoms are typical of those caused by TSWV; however, tests performed on representative symptomatic leaves with a TSWV lateral flow device (TSWV immunostrips: Agdia; hereafter referred to as immunostrip) were almost always negative. In contrast, when these leaves were tested for the tospovirus *Impatiens necrotic spot virus* (INSV), either with an INSV immunostrip or a reverse-transcription-polymerase chain reaction (RT-PCR) assay with INSV-specific primers, a positive result consistently was obtained (18). From 2006 through 2012, numerous lettuce fields in Monterey County suffered substantial economic losses from this new disease caused by INSV, as did plantings in San Benito and Santa Cruz Counties. In 2011 and 2012, this disease also occurred in the Santa Maria Valley in the south-central coast (Santa Barbara County).

Viruses in the genus *Tospovirus* (family *Bunyaviridae*), including INSV and TSWV, have spherical virions with a diameter of approximately 80 to 100 nm and an outer, host-derived lipid membrane (16). Encapsidated within these virions are three different-sized single-stranded RNAs (large [L], medium [M], and small [S]), each of which is associated with a viral-encoded nucleocapsid (N) protein (16). Tospoviruses utilize an ambisense genome strategy (i.e., some genes are encoded on the virion-sense RNA and others from the complementary-sense RNA). The L RNA encodes the RNA polymerase, which is expressed from the complementary-sense; the M RNA encodes two glycoproteins (G_n and G_c, expressed as a polyprotein from the complementary-sense and processed into mature proteins by a host protease) and an approximately 34-kDa protein involved in movement (NSm, expressed from the virion-sense); and the S RNA encodes the approximately 29-kDa N protein (expressed from the complementary-sense) and

Fig. 1. Disease symptoms induced in romaine lettuce plants by *Impatiens necrotic spot virus*. **A,** Stunted and malformed heads of plants infected early in development. **B,** Tan, necrotic spots and marginal lesions. **C,** Dark brown to black, necrotic spotting and dieback of young, developing leaves.

an approximately 52-kDa protein that is a suppressor of gene silencing (NSs, expressed from the virion-sense). Thus, the tospovirus genome has five genes (*L*, G_n/G_n, *NSm*, *N*, and *NSs*), and the sequence of the *N* gene is most commonly used for taxonomic studies of tospoviruses (16). In nature, tospoviruses are transmitted plant to plant by a relatively small number (approximately 14) of thrips species (36). The mode of transmission is persistent propagative and the virus is not transovarially transmitted. The transmission efficiencies are different among species and populations of thrips (48). Although INSV is transmitted by both *Frankliniella occidentalis* (western flower thrips [WFT]; 50) and *F. intonsa* (European flower thrips; 38), the transmission efficiency of *F. occidentalis* is much greater (38). *F. occidentalis* is a polyphagous and widespread pest of vegetables and ornamentals in fields and greenhouses in many parts of the world, including California. It also colonizes and reproduces on many weed species (6,30,31).

Because INSV is a new pathogen of lettuce in the Salinas Valley, a study was initiated to obtain information on the possible factors responsible for the emergence of this new lettuce disease and to develop disease management strategies. The objectives of this study were to document disease incidence in lettuce crops in coastal California, further characterize the pathogen, enumerate and identify potential thrips vectors associated with the disease, and investigate other local potential vegetable crop and weed reservoir hosts of INSV.

Materials and Methods

Incidence of the new INSV disease in commercial lettuce. The incidence of the new lettuce disease caused by INSV was assessed in Monterey County in 2008 (six fields) and 2009 (four fields). Fields were selected based upon grower reports of disease outbreaks, and surveys were made when fields were within 7 to 10 days of harvest. For each field, five locations (at the four corners and in the center of the field) were evaluated; at each location, the number of symptomatic plants was determined within a randomly selected area consisting of five beds (each 1 or 2 m wide by 61 m long). Mean disease incidence (percent) for each field was then calculated based on the total number of lettuce plants averaged over the five evaluated locations. Representative symptomatic plants were collected and initially tested for INSV infection with immunostrips; selected plants were also tested by RT-PCR assay with INSV-specific primers (see below).

Lettuce in the San Joaquin Valley can be affected by TSWV (T. Turini, *personal observation*). However, because symptoms in lettuce caused by TSWV and INSV are indistinguishable, lettuce fields were also surveyed in this noncoastal region of California to determine whether INSV was present. Five fields in fall 2008 and three fields in spring 2009 were assessed at weekly intervals for the incidence of tospovirus-like symptoms, as described above. Plants with tospovirus-like symptoms were collected and tested with immunostrips for both TSWV and INSV.

Detection and characterization of INSV by RT-PCR assay and DNA sequencing. To confirm the identity and assess the genetic diversity of INSV isolates collected from various plants, an RT-PCR assay was used with selected INSV primer pairs that were designed in the present study based on alignments of INSV sequences available in GenBank, unless otherwise noted (see below). Total RNA was extracted from leaf tissue with the RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. The RT-PCR assay was done with Superscript II Reverse Transcriptase (Invitrogen) and Choice Taq (Denville Scientific) according to the manufacturer's instructions. Three INSV primer pairs were designed and used in this study: INSV NSs 1409R *Hin*dIII (5′-AAGCTTATTTAAATCTAATTTAGAAATAGCTTTC-3′) and INSV NSs 63F *Xho*I (5′-CTCGAGATGTCTAGTGCAATGTATGAA ACAA-3′), which direct amplification of the complete *NSs* gene; INSV NSm 994R *Hin*dIII (5′-AAGCTTGATTTCATTATCAAA TGATATCTCTATC-3′) and INSV NSm 86F *Xho*I (5′-CTC GAGATGAATAGTTTTTTCAAATCACTCAG-3′), which direct amplification of the complete *NSm* gene; and INSV N 2021F (5′-ACACAACACAAAGCAAACCAAGCTCAAATC-3′) and N 2980c (5′-TCACGAATATAACATCATATAATCCAAAGC-3′), which direct amplification of the complete *N* gene. The PCR parameters were 94°C for 2 min; 30 cycles of 94°C for 1 min, 54°C for 50 s, and 72°C for 3 min; and a final extension of 72°C for 10 min. The PCR-amplified DNA fragments were analyzed by gel electrophoresis in 1.0% agarose, and DNA fragments of the expected size were excised from the gel with the Qiagen gel extraction kit (Qiagen). The recovered DNA fragments were cloned into the pCR-Blunt II Topo vector from the Zero Blunt Topo PCR cloning kit (Invitrogen), and sequenced as described previously (52).

Sequence alignment and phylogenetic analysis. Sequence alignments were performed with Vector NTI Version 11.0 (Invitrogen) and ClustalW in MEGA 5.1 software. Phylogenetic trees were generated using the neighbor-joining method of MEGA version 5.1, with 1,000 bootstrap replications.

Enumeration and identification of thrips recovered from lettuce plants. Lettuce plants were collected from the fields surveyed for INSV in 2008 (six fields) and 2009 (four fields) for determination of thrips populations and identification of thrips species. For each field, five plants with INSV symptoms and five plants without symptoms were collected separately from each of the five locations surveyed for a total of 25 symptomatic and 25 asymptomatic plants per field. Whole plants were harvested, placed in plastic bags, and refrigerated until processed. Thrips were recovered from whole plants using a wash method (7), in which each plant was cut and separated into individual leaves. In a sink, each leaf was washed under running tap water to dislodge all insects. The rinsate was filtered continually through a fine mesh screen. Using a camel hair brush (Cotman Series 111 number 00), all thrips from the five plants from each location were removed from the mesh screen and stored in glass vials containing 95% ethanol.

The thrips in each vial (from the five plants from each location in each field) were poured into a glass petri dish, sorted into adult and juvenile stages, and counted. Using a pipette, 75 adult thrips were removed randomly and processed for species identification. For vials with fewer than 75 thrips, approximately 50% of the total numbers of adult thrips were processed for identification, with the remainder used for RT-PCR analysis. For species identification, the body contents of adult thrips were cleared by submerging the insects for 24 h in a 2% sodium hydroxide solution. After 24 h, thrips were rinsed in distilled water for 1 h and then stored in 95% ethanol. Thrips were mounted in Hoyer's solution (41) on a glass slide and examined with a compound microscope. Identification of thrips to species was made based on morphological features according to published keys (14,15,25,26). The remaining thrips were stored in 95% ethanol and used for RT-PCR assays (see below). In addition, iceberg lettuce samples from Huron, CA (Fresno County, San Joaquin Valley) were collected in 2009 and thrips populations and species determined as described above.

Association of thrips populations and disease incidence. To investigate relationships between thrips numbers and INSV disease incidence, thrips data were further analyzed for fields grown under a standard Salinas Valley production system (i.e., conventional, direct-seeded romaine grown on 2-m-wide beds). The REG procedure was used to evaluate the relationship between total mean number of thrips using the RSQUARE model selection method in SAS (version 9.3; SAS Institute). The regression was conducted on the total mean number of thrips (averaged across the number of INSV-symptomatic and -asymptomatic plants) and the INSV incidence for the six field locations where romaine lettuce was grown conventionally from seed on 2-m-wide beds. The rationale for restricting the regression analysis to these six fields was to remove potential effects of lettuce type (romaine versus greenleaf), planting method (seeded versus transplanted), bed width (2 versus 1 m), or production system (conventional lettuce monoculture versus organic lettuce interplanted with sweet alyssum [*Lobularia maritime*], a beneficial insectary plant [1,4] that attracts natural enemies of aphids) that differed for the four surveyed fields that were ex-

cluded (Salinas SpEB and Gonzales S10 in 2008 and Salinas SpEB and Gonzales NIv in 2009).

Development of an RT-PCR assay for detection of INSV in thrips. An RT-PCR assay for detection of INSV in thrips was developed in the Gilbertson laboratory as part of this study. Thrips collected during the course of this study were stored in 95% ethanol prior to use in the RT-PCR assay; thus, the first step of the assay was to vacuum-dry the thrips. Total RNA was extracted from the thrips with the Qiagen RNAeasy kit; this RNA was used in the RT-PCR assay with the INSV *N* gene-specific primers described above. In preliminary experiments, it was determined that high-quality RNA (i.e., without degradation or PCR inhibitors) could be extracted from 1 to as many as 200 thrips with the Qiagen RNAeasy kit (*data not shown*). The amount of RNA used in the INSV–thrips RT-PCR assays ranged from 50 to 500 nm, and detection of INSV was based upon amplification of the target 960-bp *N* gene DNA fragment (as described above for detection of INSV from leaf tissue). In these experiments, the amount of RNA in each reaction was not equalized because the objective was simply to determine the presence or absence of the virus in the thrips samples. It is also important to note that detection of the virus in thrips does not necessarily prove that the insects were viruliferous.

In the first experiment, thrips collected from lettuce plants with and without INSV symptoms from three fields surveyed in 2008 (Gonzales S7N in April, Gonzales S10 in June, and Soledad D17A in July) were tested for INSV with the RT-PCR assay. The number of thrips used in this experiment was 40 to 580 thrips/field. In a second experiment, thrips were collected from an organic romaine field that was interplanted with sweet alyssum. Thrips were collected from lettuce plants with and without INSV symptoms, and from the flowers of sweet alyssum plants. Thrips were treated as described above, and the RNA was extracted and used in the RT-PCR assay. Sweet alyssum is not reported to be a host of INSV and, consistent with these reports, the plants in this field did not show disease symptoms. However, to test the sweet alyssum plants for INSV, three composite samples of 25 plants each were collected and tested for INSV infection with the RT-PCR assay.

Field surveys for potential vegetable crop and weed reservoirs of INSV. Field surveys were conducted in the Salinas Valley from 2007 to 2011 to identify and collect potential INSV reservoir hosts. Survey sites were selected based on close proximity to outbreaks of INSV in lettuce fields. Non-lettuce vegetable fields established adjacent to or near lettuce fields with documented INSV outbreaks were examined for plants with virus-like symptoms.

Plants with symptoms were collected and tested for INSV using immunostrips and, in some cases, the RT-PCR assay with the *N* gene primer pair. Weeds growing in or near these lettuce fields were also surveyed. If vegetable crop or weed plants showed no obvious disease symptoms, randomly selected asymptomatic plants representing the most predominant species were collected. Crop and weed plants were collected and bulked together by species, placed into plastic bags, and kept refrigerated until testing. Each sample consisted of a composite of leaves taken from five plants. In 2008 and 2009, weed surveys were also conducted in and around lettuce fields in the lettuce-growing area of Fresno County, where sites were selected based on documented tospovirus disease outbreaks in the lettuce fields.

Surveys of coastal lettuce production areas conducted in 2009 indicated that, of the weeds tested, shepherd's purse (*Capsella bursa-pastoris*) and little mallow (*Malva parvifolia*) were most commonly infected with INSV. Thus, these weeds were emphasized for collection from 2009 to 2011. In addition, because little mallow is a common weed species that persists year-round in parts of the Salinas Valley where INSV outbreaks were documented, two winter surveys of little mallow and other weeds were conducted in vineyards, along roadways, and in fallow fields without lettuce.

Sap transmission of INSV. To further assess the properties of the virus and confirm that little mallow is a host of INSV, sap transmission experiments were conducted as previously described (52). Three sets of 10 *Nicotiana benthamiana* plants and one set of 10 little mallow plants were used; little mallow plants were grown from seeds collected from plants growing in Monterey County. INSV-infected faba bean and lettuce leaf tissues were collected from the Salinas Valley and confirmed to be infected with INSV (and not TSWV) with immunostrips. Sap was prepared by grinding leaves in ice-cold 0.01 M potassium phosphate buffer (pH 7.0) containing 0.2% sodium sulfite. The sap was rubbed onto celite-dusted leaves of young *N. benthamiana* (four- to six-true-leaf stage) and little mallow (two-true-leaf stage) plants. The *N. benthamiana* plants were observed for symptoms beginning 5 days post inoculation (dpi), whereas the inoculated little mallow plants were observed beginning 10 dpi; all plants were observed until symptoms appeared or up to 30 dpi.

Results

Incidence of the new INSV disease in commercial lettuce. In 2008, disease incidence in the six surveyed lettuce fields was 0.5 to 27.0% whereas the incidence in 2009 was 1.0 to 5.6% in the four surveyed fields (Table 1). For all the fields, no other viral diseases were detected. Consistent with these observations, representative symptomatic plants from all of these fields tested positive for INSV with immunostrip and RT-PCR assays. In general, the greatest incidences of INSV symptoms were observed later in the season (e.g., July and August 2008 and September 2009; Table 1). In addition to these surveys, growers and other field personnel submitted numerous iceberg, redleaf, and butterhead lettuce plants with tospovirus-like disease symptoms collected from fields in coastal production areas from 2008 to 2012; these plants also tested positive for INSV by immunostrips or RT-PCR assays (*data not shown*). Together, these results indicate that INSV was responsible for the tospovirus-like disease symptoms observed in the coastal California lettuce-growing areas, and provide evidence that this virus has emerged as an important lettuce pathogen in this region.

In fall 2008 and spring 2009, five and three lettuce fields, respectively, in the San Joaquin Valley were surveyed for tospovirus disease symptoms and thrips. The percent incidence of lettuce plants (iceberg and romaine) showing tospovirus-like symptoms in these fields was 0 to 1.3% in the fall, whereas no lettuce plants with tospovirus-like symptoms were detected in the monitored fields in the spring (*data not shown*). Representative symptomatic plants tested positive for TSWV and negative for INSV based on immunostrip tests. Lettuce plants from the San Joaquin Valley with symptoms of tospovirus infection were also collected from additional fields during surveys conducted from 2008 to 2012. Based on immunostrip tests, all of these samples tested positive for TSWV, whereas none tested positive for INSV. Thus, in contrast to the coastal production area, the only detected tospovirus infecting lettuce in the San Joaquin Valley was TSWV.

Detection and characterization of INSV by RT-PCR assay. A representative INSV isolate (SV-L1 from Gonzales, CA, collected in 2007), obtained from lettuce leaves having typical tospovirus symptoms and testing positive with INSV immunostrips, was used for molecular characterization. In RT-PCR analyses performed with the NSs, NSm, and N primer pairs, the expected-size DNA fragments, approximately 1,350, 900, and 960-bp, respectively, were amplified. The sequences of the SV-L1 *NSs* (GenBank accession number KF745142), *NSm* (KF745141), and *N* genes (KF745140) were 98 to 99% identical to those of other INSV isolates from a range of host plants from the United States as well as those from other countries (Table 2). These results indicate that the INSV isolates from lettuce in California were nearly identical to isolates from other hosts and geographic locations. Thus, subsequent comparisons were performed with the *N* gene sequence only, which is typically used for characterization of tospoviruses.

All lettuce samples that tested positive for INSV infection with the immunostrips also tested positive in the RT-PCR assay with the INSV *N* gene primer pair (*data not shown*). The *N* gene sequences of three additional lettuce isolates of INSV from romaine (Beck isolate from 2007), greenleaf (Gonzales isolate from 2007), and

redleaf types (Benedix isolate from 2007) were determined and were 100% identical to each other and to the *N* gene sequence of the SV-L1 isolate. These results suggest there is little genetic diversity among the INSV isolates infecting lettuce in the Salinas Valley. The expected-size *N* gene fragment also was amplified from other plant species infected with INSV (based on a positive result with the INSV immunostrips), including artichoke (*Cynara cardunculus* var. *scolymus*), bell pepper (*Capsicum annuum*), faba bean (*Vicia faba*), little mallow, *Phalaenopsis* orchid, radicchio (*Cichorium intybus*), snapdragon (*Antirrhinum majus*), and spinach (*Spinacia oleracea*). All of these plants had typical tospovirus symptoms (stunting, with chlorotic and necrotic leaf spots), except artichoke and little mallow, which were symptomless. Sequence analysis confirmed that there were *N* gene fragments for artichoke (KF926825), basil (KF926827), bell pepper (KF926832), *Phalaenopsis* orchid (KF926828), radicchio (KF926829), snap-

Table 1. *Impatiens necrotic spot virus* (INSV) disease incidence, thrips populations from symptomatic and asymptomatic lettuce plants, and thrips species identified in surveyed lettuce fields in the Salinas Valley (Monterey County) of California in 2008 and 2009

			INSV		Mean number (%) of thrips per five plants[a]			Thrips species (% of total)[b]		
Field	**Month, year**	**Lettuce type**	**Incidence (%)[c]**	**Symptoms**	**Total**	**Adults**	**Juveniles**	***F. occidentalis***	***T. tabaci***	**Other[d]**
Gonzales S7N	April 2008	Romaine	2.3	Yes	62	34 (54)	28 (46)	86	11	3
	…	…	…	No	90	56 (65)	34 (36)	…	…	…
Gonzales ShR	June 2008	Romaine	1.0	Yes	58	45 (81)	13 (19)	97	0	3
	…	…	…	No	34	27 (79)	7 (21)	…	…	…
Salinas SpEB	June 2008	Romaine	3.0	Yes	312	168 (53)	144 (47)	93	4	3
	…	…	…	No	316	177 (56)	139 (44)	…	…	…
Gonzales S10	June 2008	Greenleaf	0.5	Yes	211	20 (10)	191 (90)	94	4	2
	…	…	…	No	282	68 (24)	214 (76)	…	…	…
Soledad D17A	July 2008	Romaine	27.0	Yes	611	559 (91)	52 (9)	98	0	2
	…	…	…	No	370	328 (89)	42 (11)	…	…	…
Soledad CGD	August 2008	Romaine	14.8	Yes	236	207 (88)	29 (12)	98	2	0
	…	…	…	No	66	60 (91)	6 (9)	…	…	…
Salinas SpEB	June 2009	Romaine	1.0	Yes	386	92 (24)	294 (76)	84	13	3
	…	…	…	No	327	91 (28)	236 (72)	…	…	…
Gonzales NIv	August 2009	Greenleaf	5.1	Yes	86	82 (95)	4 (5)	93	4	3
	…	…	…	No	82	82 (100)	0 (0)	…	…	…
Gonzales D7A	August 2009	Romaine	2.0	Yes	38	38 (100)	0 (0)	95	0	5
	…	…	…	No	51	50 (98)	1 (2)	…	…	…
Soledad S1	September 2009	Romaine	5.6	Yes	95	90 (94)	5 (6)	97	1	2
	…	…	…	No	90	82 (9)	8 (9)	…	…	…

[a] Groups of symptomatic and asymptomatic plants were harvested, placed in plastic bags, and kept refrigerated during transit and until processing. In a sink, each plant was cut and separated into individual leaves, with each leaf then held under running tap water to dislodge all insects. The rinsate was filtered continually through a fine mesh screen. Thrips were removed from the mesh screen and stored in vials containing 95% ethyl alcohol. Vial contents were poured into a glass petri dish and thrips adults and juveniles were counted.

[b] Species: *Frankliniella occidentalis, Thrips tabaci*, and Other. Represents results of identification of 75 adult thrips (or approximately 50% of the total numbers of collected thrips if <75 thrips) per field. Insects were cleared by submerging in a 2% sodium hydroxide solution for 24 h, rinsing in distilled water for 1 h, and storing thrips in 95% ethyl alcohol until mounted in Hoyer's solution (41) on a glass slide. Thrips were identified to species based on morphological features according to published keys (14,15,25,26).

[c] Disease incidence was determined by selecting five locations per field (at the four corners and in the center). For each location, the number of plants showing symptoms was counted in an area of five beds (each 2 m wide by 61 m long). Mean disease incidence (%) was calculated based on the total number of lettuce plants in each area surveyed.

[d] Other thrips species identified included *Aeolothrips* spp., *Ankothrips* spp., *Chirothrips* spp., *Frankliniella insularis*, *F. minuta*, and *Thrips australis*.

Table 2. Comparison of the sequences of the nucleocapsid (*N*), *NSm*, and *NSs* genes of an isolate (SV-L1) of *Impatiens necrotic spot virus* (INSV) from a diseased romaine lettuce plant in the Salinas Valley (Monterey County) of California collected in 2007, with sequences of other INSV isolates[a]

Gene	**Identity with SV-L1 (%)**	**Geographic location of isolate**	**Host plant**	**GenBank accession number**
N	99	United States (Washington State)	*Solanum tuberosum*	HM802206.1
	99	The Netherlands	*Impatiens* sp.	X66972.1
	98	China	*Dendrobium* sp.	FN400773.1
	98	China	*Oncidium* sp.	FN400772.1
	98	China	*Hymenocallis littoralis*	GU112504.1
	98	Japan	(*data not published*)	AB109100.1
	98	Italy	*S. lycopersicum*	DQ425096.1
NSm	99	China	*H. littoralis*	GU112503.1
	99	China	(*data not published*)	GQ336990
	98	Italy	*S. lycopersicum*	DQ425095.1
NSs	99	The Netherlands	*Impatiens* sp.	X66972.1
	99	United States (Washington State)	(*data not published*)	EU095193.1
	99	China	*Oncidium* sp.	FN400772.1
	99	China	*Dendrobium* sp.	FN400773.1
	99	Japan	(*data not published*)	AB109100.1
	98	Italy	*S. lycopersicum*	DQ425096.1
	98	China	(*data not published*)	GQ33698.1
	98	China	*H. littoralis*	GU112504.1

[a] Based upon complete sequences of these genes. The *N* gene is on the small (S)-RNA of INSV and encodes the N protein, which binds the viral RNAs and forms nucleocapsids that are a major constituent of the virions (16). *NSm* = nonstructural protein gene on the medium (M)-RNA of INSV, which encodes NSm, a putative movement protein (16). *NSs* = nonstructural protein gene on the S-RNA of INSV, which encodes the NSs protein, a suppressor of gene silencing (16).

dragon (KF926831), and spinach (KF926830), and revealed 99% identity with *N* gene sequences of the INSV isolates from the Salinas Valley lettuce samples. Together, these results indicated that the INSV isolates infecting lettuce in the Salinas Valley are genetically homogenous and are closely related to INSV isolates infecting other hosts in the Salinas Valley and other geographical regions.

To further investigate the relationship among the INSV isolates from lettuce in the Salinas Valley and INSV isolates from other geographical regions, a phylogenetic tree was constructed with the *N* gene sequences (Fig. 2). The INSV isolates were placed into two groups, one with isolates from the United States (Salinas Valley and one isolate from North Carolina), and one isolate from The Netherlands; and the other with isolates from Asia (China and Japan) and an isolate from Italy. This analysis revealed grouping of isolates according to geographic origin but also evidence of long-distance spread of INSV. In the latter case, such spread may have occurred via infected ornamental plants, because INSV is an important pathogen of flowers and other greenhouse-grown plants (9).

Thrips populations recovered from lettuce. Thrips were recovered consistently from symptomatic and asymptomatic lettuce plants from the surveyed fields. Populations varied from field to field and year to year. For the six fields surveyed in 2008, the number of thrips recovered from symptomatic plants was 58 to 611 thrips per five-plant sample (mean = 248), whereas 34 to 370 thrips per five-plant sample (mean = 193) were collected from asymptomatic plants (Table 1). For the four fields surveyed in 2009, the number of thrips recovered from symptomatic and asymptomatic plants was 38 to 386 (mean = 151) and 51 to 327 (mean = 137) thrips per five-plant sample, respectively (Table 1). In general, the numbers of thrips recovered from symptomatic versus asymptomatic plants from the same field were similar, with two exceptions in 2008: in the Soledad D17A field, 611 thrips were recovered from symptomatic plants, whereas 370 were collected from asymptomatic plants; and in the Soledad CGD field, 236 thrips were collected from symptomatic plants, whereas 66 were collected from asymptomatic plants (Table 1). Interestingly, these were the two fields with the greatest overall incidences of INSV symptoms: 27.0% in Soledad D17A and 14.8% in Soledad CGD (Table 1).

The majority of thrips recovered from lettuce plants were adults, although juveniles were also present in most collections (Table 1). Two notable exceptions were the Gonzales S10 field in 2008, in which 90 and 76% of the thrips collected from symptomatic and asymptomatic plants, respectively, were juveniles; and the Salinas SpEB field in 2009, in which 76 and 72% of the thrips collected from symptomatic and asymptomatic plants, respectively, were juveniles (Table 1). In general, the greatest proportion of juvenile thrips were detected earlier in the growing season in both years (e.g., April, June, and July, compared with August and September; Table 1).

Identification of thrips recovered from lettuce. The majority of thrips identified from the 10 lettuce fields surveyed in coastal California in 2008 and 2009 were *F. occidentalis*, the WFT. WFT comprised 84 to 98% of the thrips identified from plants in these lettuce fields (Table 1). The second most commonly identified

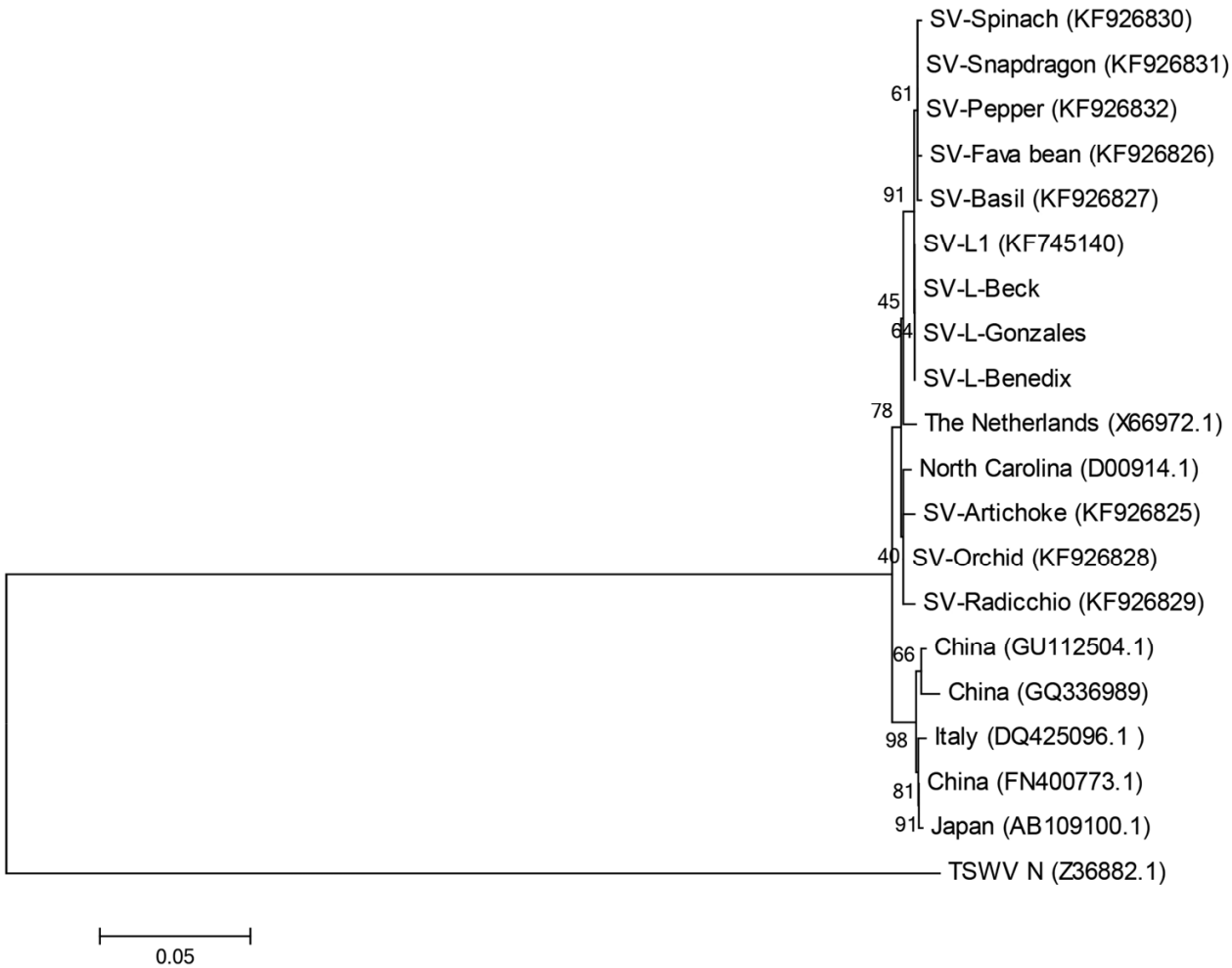

Fig. 2. Phylogenetic consensus tree showing the relationship of *Impatiens necrotic spot virus* isolates from various hosts and geographic locations based on an alignment of complete nucleocapsid (*N*) gene sequences. Analyses were performed with MEGA 5.1 using the neighbor-joining method. The scale bar indicates the length of horizontal branches corresponding to the rate of substitution/nucleotide. The *Tomato spotted wilt virus* (TSWV) *N* gene sequence was used as an outgroup. SV designates isolates collected during this study from the Salinas Valley, CA; SV-L1, SV-L-Beck, SV-L-Benedix, and SV-L-Gonzales were collected from lettuce.

species was *Thrips tabaci*, the onion thrips, which comprised 0 to 13% of the total thrips identified. Small numbers of other species also were identified, including *Aeolothrips* spp., *Ankothrips* spp., *Chirothrips* spp., *F. insularis*, *F. minuta*, and *T. australis* (Table 1). The WFT was also the predominant species recovered in 2009 from iceberg lettuce plants with tospovirus symptoms in the Huron region of Fresno County. In that sample, 95% of thrips collected from lettuce plants infected with TSWV were WFT.

Association of thrips populations and disease incidence. For six romaine lettuce fields grown in the conventional direct-seeded manner on 2-m-wide beds, a positive and significant correlation (r^2 = 0.91, P = 0.003) between numbers of thrips recovered from lettuce plants and incidence of INSV was detected via a fitted regression line (Fig. 3). Because of insufficient data, such calculations were not made for direct-seeded greenleaf (one field) and transplanted (1-m-wide beds) organic romaine (two fields).

Detection of INSV in thrips with an RT-PCR assay. For the Gonzales S7N romaine lettuce field (first experiment), INSV was detected in four of five groups (vials) of thrips collected from symptomatic plants and in three of five groups of thrips from asymptomatic plants. For the Gonzales S10 greenleaf lettuce field, INSV was detected in all five groups of thrips collected from symptomatic plants and in none of the five groups from asymptomatic plants. Similar results were obtained with thrips collected from the Soledad D17A romaine lettuce field, with INSV detected in four of five groups of thrips from symptomatic plants and in zero of five groups from asymptomatic plants. In the Salinas SpEB organic romaine field (second experiment), INSV was detected in three of five groups of thrips collected from symptomatic lettuce plants and zero of five groups from asymptomatic plants (Fig. 4). Interestingly, INSV was detected in two of five groups of thrips collected from asymptomatic sweet alyssum interplanted with lettuce as a beneficial insectary crop, whereas the sweet alyssum plants from which the thrips were collected always tested negative for INSV (Fig. 4).

Field surveys for potential vegetable and weed reservoirs of INSV. The three surveyed fields of radicchio exhibited severe tospovirus-like disease symptoms (stunted and distorted growth, chlorotic leafspots, and mottling and necrosis of leaves). All representative leaf samples from radicchio plants with these symptoms tested positive for INSV with immunostrips and the RT-PCR assay. Surveys of three broccoli (*Brassica olearacea* var. *italica*) and four artichoke fields revealed no virus-like symptoms. Randomly collected samples (11 composite samples from the three broccoli fields and 39 composite samples from the four artichoke fields) all tested negative for INSV with immunostrips.

Of the plant samples received from farmers, pest control advisors, and other field personnel from 2007 to 2009, INSV was detected by immunostrips and RT-PCR assays in the following plants with tospovirus-like symptoms (some combination of foliar chlorosis, mottling, necrosis, and ringspots): basil (*Ocimum basilicum*), bell pepper, calla lily (*Zantedeschia aethiopica*), celery (*Apium graveolens*), faba bean, radicchio, and spinach. Most of these samples came from fields in the Salinas Valley that were near lettuce fields with INSV-infected plants. Exceptions were samples of bell pepper and spinach infected with INSV from a greenhouse and field, respectively, in San Benito County and INSV-infected basil from a greenhouse in Santa Cruz County. These two counties are geographically distant from each other and from the Salinas Valley lettuce-growing area.

In the 5-year weed survey, the weeds most commonly found infected with INSV were little mallow (70 positive composite samples per 290 total composite samples tested) and shepherd's purse (31 positive composite samples per 126 total composite samples tested) (Table 3). INSV infection was detected at low incidences in 11 other weeds species (Table 3). Of all 13 INSV-infected weeds found in these surveys, only annual sowthistle, hairy nightshade, and London rocket showed virus-like symptoms, including chlorotic and necrotic leafspots and blotches. In these surveys, a number of other weed species tested negative for INSV: *Convolvulus arvensis* (field bindweed), *Cyperus esculentus* (yellow nutsedge), *Foeniculum vulgare* (fennel), *Heliotropium curassavicum* (alkali heliotrope), *Lepidium coronopus* (swine cress), *Medicago polymorpha* (California burclover), *Poa annua* (annual blue grass), *Polygonum aviculare* (knotweed), *Raphanus sativus* (wild radish), *Rumex crispus* (curly dock), *Stellaria media* (common chickweed), *Taraxacum officinale* (common dandelion), *Tribulus terrestris* (puncture vine), and other single-plant samples of unidentified species.

Sap transmission of INSV. All 30 (three sets of 10 plants each) *N. benthamiana* plants rub inoculated with sap prepared from INSV-infected lettuce plants developed ringspots in the inoculated leaves by 5 dpi, followed by chlorosis, mottling, and epinasty of newly emerged leaves. Eventually, these plants developed systemic necrosis and died. For the single set of 10 little mallow plants rub inoculated with sap prepared from INSV-infected faba bean leaves, inoculated leaves of 4 plants developed chlorotic leaf spots at 10

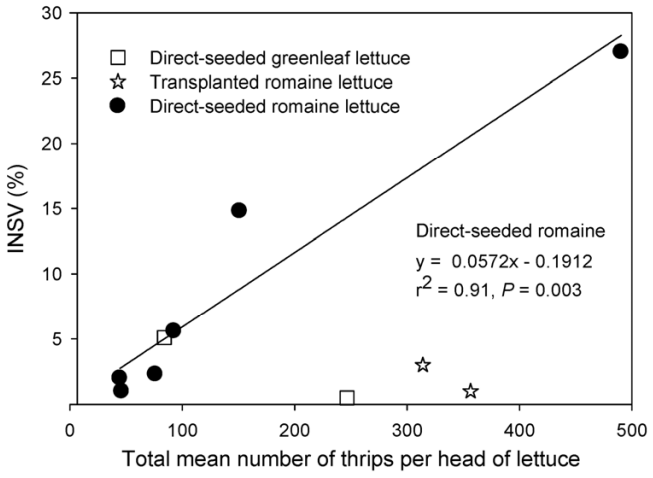

Fig. 3. Relationship between the number of thrips recovered from lettuce plants and the incidence of *Impatiens necrotic spot virus* (INSV) for lettuce crops in the Salinas Valley of California in 2008 and 2009. The fitted linear regression line corresponds to six fields of direct-seeded, conventional romaine lettuce. The regression analysis was restricted to the six fields having the same type of lettuce (romaine) and planting method (direct-seeded). There were insufficient data to evaluate the relationships with transplanted romaine or greenleaf lettuce.

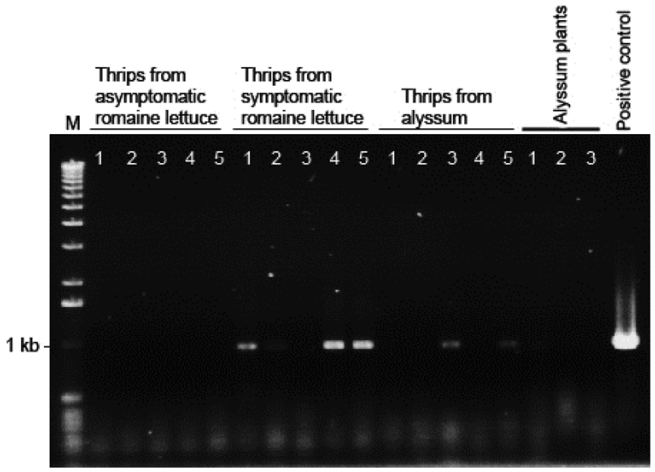

Fig. 4. Reverse-transcription polymerase chain reaction (RT-PCR) assay for detection of *Impatiens necrotic spot virus* (INSV) in thrips collected from asymptomatic and symptomatic lettuce plants, in thrips collected from asymptomatic sweet alyssum interplanted with lettuce as a beneficial insectary crop, and in asymptomatic sweet alyssum plants in a field in the Salinas Valley, California. INSV-infected lettuce leaf tissue was used as the positive control sample and healthy lettuce leaf tissue was used as the negative control sample (*not shown*). Lane 1 is the 1-kb DNA ladder (M, Invitrogen). Details of the RT-PCR assay are described in the main text.

dpi, and some of these spots later developed necrotic centers. Furthermore, although obvious symptoms did not develop in newly emerged leaves of any of the little mallow plants, evidence of systemic infection of the four plants that showed symptoms in inoculated leaves came from detection of INSV by the RT-PCR assay with the *N* gene primer pair. Interestingly, although the distribution of INSV in leaves of systemically infected plants was irregular (i.e., the virus was not detected in all leaves of the tested plants), the virus consistently was detected in the flowers of these plants. In general, symptoms induced by INSV in inoculated leaves and systemically infected plants were considerably more severe in *N. benthamiana* than in little mallow plants, and this was consistent with the greater rates of infection detected in *N. benthamiana* (100%) compared with little mallow following sap inoculation (40%). However, attempts to infect little mallow plants with sap prepared from INSV-infected *N. benthamiana* plants were unsuccessful, indicating that the INSV titer may have been low or the virus inactivated in sap prepared from *N. benthamiana* plants.

Discussion

The emergence of a new disease of lettuce in coastal California caused by the thrips-transmitted tospovirus INSV is a significant concern for this important industry because INSV-infected lettuce plants are not marketable due to stunted growth as well as necrotic and distorted leaves. Although first described in this region in 2006, the results of this survey revealed that INSV has become established in the lettuce production region of the Salinas Valley and has reoccurred, to some extent, every year. Moreover, disease incidences reached as much as 27% in individual fields, which resulted in substantial economic loss. The disease is also spreading in the coastal region of California, because lettuce from the Santa Maria Valley (Santa Barbara County) was confirmed to be infected with INSV in 2011 and 2012 (*data not shown*). Although INSV has previously been reported to infect lettuce grown in greenhouses in Pennsylvania (13) and in fields in Italy (45,46), the virus was not considered a major problem on lettuce. The emergence of the disease in a major lettuce-producing region such as the Salinas Valley demonstrates an important change in the impact and host range of the virus.

The reasons for the recent emergence of this lettuce disease caused by INSV in California are not clear. INSV has been present in the Salinas Valley and other coastal areas for many years, and has been documented infecting ornamentals in greenhouses and container-grown field facilities, including some close to lettuce fields. It is unknown why INSV from infected ornamental plants did not infect lettuce prior to 2006. Furthermore, emergence of INSV was not detected in winter and early spring lettuce crops in California's Central Valley or desert regions during this 5-year study (2006 to 2011). In these two noncoastal regions, all tospovirus-like symptoms found in lettuce were caused by TSWV.

One possible explanation for the recent outbreaks of INSV in lettuce is the emergence of a novel INSV strain that is particularly virulent on lettuce. However, the findings in this study that (i) the sequences of the *NSs*, *NSm*, and *N* genes of a representative INSV isolate (SV-L1) from lettuce were almost identical (98 to 99% identities) to those of previously characterized INSV isolates from ornamentals and vegetables (18,19) and (ii) the *N* gene sequences of four lettuce-infecting INSV isolates from the Salinas Valley were 100% identical suggest that the INSV isolates infecting lettuce in this region are not new or variant strains. This is further supported by the finding that the *N* gene sequences of INSV isolates from lettuce were >98% identical to those of isolates from ornamental and vegetable plant hosts from the Salinas Valley and other geographically diverse locations. Thus, it appears more likely that ornamentals or other crops in the Salinas Valley were the sources of INSV infecting lettuce in the coastal area. This conclusion also is supported by the finding that tospovirus symptoms on lettuce in inland lettuce production regions, where there is no extensive commercial ornamental production, were associated with TSWV rather than INSV.

Another explanation for the emergence of INSV in lettuce in the Salinas Valley is a change in the thrips population dynamics or species composition, such as the introduction of a new thrips species that facilitated the spread of INSV to lettuce. For example, a new thrips vector of INSV, *F. fusca*, was reported in Georgia and was thought to be involved in an expansion of the host range of the virus (27,38). However, the results of thrips collections and species identifications in this California study revealed that the majority of thrips on lettuce plants collected in the Salinas Valley were WFT, the most common thrips species in the Salinas Valley and other parts of California and an efficient vector of INSV. Furthermore, WFT is a well-established pest of lettuce and other crops in the Salinas Valley (7,51). Thus, although it does not appear that the introduction of a new thrips species to the Salinas Valley is responsible for the INSV outbreaks, it is possible that increased populations or other changes in WFT behavior could explain the INSV outbreaks in lettuce. Indeed, outbreaks of tospoviruses in other California vegetables have been increasing (e.g., TSWV in pepper and tomato [*Solanum lycopersicum*] and *Iris yellow spot virus* in leek [*Allium ampeloprasum*] and onion [*A. cepa*]), which have been attributed to increased thrips populations. The existence in the Salinas Valley of WFT biotypes or sympatric cryptic species (37), and possible differences in virus transmission efficiency of such subspecies, may also play a role; however, this possibility has not yet been investigated.

The majority of the romaine lettuce grown in the Salinas Valley is direct-seeded into 2-m-wide beds. The finding of a positive and significant correlation between thrips populations and incidence of INSV in these fields indicated that a minimum threshold population may be required for severe incidences of disease in this type of lettuce production. Furthermore, this suggests that thrips management with appropriately timed insecticide sprays may be an effective approach for keeping INSV incidences below damaging thresholds. It will be important to determine whether a similar correlation exists for other types of lettuce and lettuce production systems. The finding that the highest incidence of disease was in fields planted later in the season suggested that a build-up of populations of viruliferous adult thrips may also be important in disease development. Most of the thrips recovered from the monitored fields were adults, regardless of whether plants had symptoms or not. However, finding both adult and juvenile thrips on lettuce plants in all but 2 of the 10 fields indicated that thrips reproduced on lettuce, and suggests that infected lettuce can serve as a source of viruliferous thrips for secondary spread of the virus in lettuce

Table 3. Weeds collected from lettuce growing areas in the Salinas Valley of California from 2007 to 2011 that tested positive for *Impatiens necrotic spot virus* (INSV)

Weed species (common name)[a]	Symptoms[b]	INSV-positive/ total[c]
Amsinckia menziesii (fiddleneck)	None	1/13
Capsella bursa-pastoris (shepherd's purse)	None	31/126
Chenopodium album (lambsquarters)	None	2/13
C. murale (nettleleaf goosefoot)	None	1/11
Conyza bonariensis (flax leaved fleabane)	None	2/12
Erodium sp. (filaree)	None	1/13
Hirschfeldia incana (shortpod mustard)	None	2/14
Lactuca serriola (prickly lettuce)	None	2/7
Malva parvifolia (little mallow)	None	70/290
Matricaria camomilla (pineappleweed)	None	2/14
Sisymbrium irio (London rocket)	Chl-S	1/12
Solanum sarrachoides (hairy nightshade)	Chl-B, Nec	3/15
Sonchus oleraceus (annual sowthistle)	Nec	1/12

[a] Weeds were collected in or near lettuce fields where INSV outbreaks had been documented. Plants were collected and grouped by species.

[b] Symptom abbreviations: Chl-S = chlorotic streaks, Chl-B = chlorotic blotches, and Nec = necrotic spots.

[c] Number of INSV-positive composite samples/total samples tested. Weeds were sorted into composite samples of five plants; leaves were then removed from the five plants and used for INSV immunostrip testing as described in the main text.

fields. This possibility was supported by results of the RT-PCR assays of thrips, in which INSV was detected more frequently in thrips collected from symptomatic than asymptomatic plants. Together, these results indicate that monitoring thrips populations in lettuce will be an important tool in timing thrips management practices, and that the RT-PCR assay for detecting INSV in thrips could be used to further refine when and where to implement these practices. For example, the RT-PCR assay could reveal the presence of the virus in thrips collected from INSV-host crops before the appearance of disease symptoms, or in thrips collected from non-INSV-host plants acting as thrips reservoir habitats that would not develop symptoms (e.g., sweet alyssum).

Weeds may play an important role in the epidemiology of the INSV disease of lettuce in coastal California. Studies of INSV in greenhouses in Massachusetts revealed that the virus infects a wide diversity of weeds (49). In Georgia, yellow and purple nutsedge (*C. esculentus* and *C. rotundus*, respectively), important weed species, are hosts for INSV (22). Furthermore, a number of studies have demonstrated the role of weeds as reservoirs of INSV in greenhouses and fields (31). Thus, it was not surprising that this 5-year survey revealed 13 weed species infected with INSV. Although most of these weed species were infected at very low incidences and may not be important epidemiologically, two species that persist year-round in the central coast region, little mallow and shepherd's purse, were commonly infected and may be important reservoirs. Although other weeds tested negative for INSV, the sample size for some of these species was relatively small. Thus, additional sampling and testing is needed in order to conclude that all of these weeds are not hosts of INSV and, thus, play no role in disease epidemiology.

In the Salinas Valley, lettuce is present in the field as multiple overlapping crops from January to November, with a 2-week lettuce-free period only in December. INSV-infected weeds could serve as virus reservoirs and act as sources of primary inoculum for lettuce planted in early spring, as well as maintaining the virus between the summer- and fall-planted crops. Little mallow is a weed that, under coastal California conditions, can grow as a biennial or perennial (10). These properties, together with the prevalence of INSV infection, may make this weed a particularly important INSV reservoir in coastal California, especially during the lettuce-free period. In a number of places in the Salinas Valley, INSV outbreaks have occurred in lettuce fields next to or near vineyards that harbored large populations of perennial *Malva* plants. Moreover, the winter surveys in this study confirmed INSV infections in a number of these *Malva* plants. The fact that little mallow, shepherd's purse, and most of the other INSV-infected weeds collected in the field were symptomless complicates the use of rogueing these reservoir hosts as a management strategy for INSV. However, the weed management component of the LMV integrated pest management (IPM) program in the Salinas Valley (12), which targets weeds regardless of the species or presence or absence of virus symptoms, should help in the management of INSV. The epidemiology of INSV disease in lettuce is complex; therefore, understanding the precise role of weeds requires additional research. Some factors that could be involved in INSV outbreaks include host preferences of the thrips vectors, and acquisition and transmission efficiencies of the vectors.

Historically, INSV was considered to be a pathogen primarily of ornamentals (9). However, the survey in this study indicated that INSV can infect and cause disease in a large number of vegetable crops in addition to lettuce, including basil, bell pepper, celery, faba bean, radicchio, and spinach. Faba bean is also often included as a legume component in legume-cereal cover crops in the coastal region (2,3). For basil, celery, faba bean, bell pepper, and radicchio, this is the first report of INSV infecting these crops in California. Liu et al. (20) recently reported that field-grown spinach in the Salinas Valley was infected with INSV; the finding in this study of spinach infected with INSV in a San Benito County greenhouse is consistent with that report. Moreover, these findings are part of a worldwide trend in which the host range for INSV is expanding beyond ornamentals into vegetable and other crops. INSV recently has been reported to infect basil (34), blackberry (*Rubus* spp.; 43), peanut (*Arachis hypogaea*; 32,47), pepper (11,28), potato (*S. tuberosum*; 8,33,35), tobacco (*N. tabacum*; 21,23), tomatillo (*Physalis ixocarpa*; 11), tomato (39), and other crops (46).

The California lettuce industry faces a significant challenge in managing tospovirus diseases of lettuce, including INSV in the coastal region and TSWV in the inland counties. Currently, tospovirus-resistant lettuce cultivars are not available. Thus, growers should follow a rigorous sanitation program that includes managing weeds and promptly plowing and destroying crop residues in harvested lettuce fields. These measures will reduce inoculum sources and thrips habitats. Monitoring and mapping outbreaks of INSV could provide insight into areas with populations of perennial weeds such as little mallow that are reservoirs of INSV. This information could also provide growers with an indication of fields with a risk of the disease, because it has been observed that INSV outbreaks tend to occur in certain locations in California. As previously mentioned, monitoring and managing the thrips vector is an important management strategy. However, WFT management with insecticides is difficult due to limitations in efficacy of currently available insecticides, challenges in applying materials to plant structures where thrips tend to aggregate (e.g., flowers), development of insecticide resistance in WFT, high reproduction rate and multiple generations of the insect, and strong north-to-south Salinas Valley winds that reintroduce thrips to fields on a daily basis (24).

Finally, growers should carefully consider which noncrop plant species to grow on lettuce ranches. For example, ground covers are sometimes planted along roads adjacent to fields in order to reduce dust. If possible, growers should choose ground covers that are not hosts of INSV or major reservoirs of thrips. A survey of one such ground cover, iceplant (*Carpobrotus* spp.), found that the flowers harbored an average of 51 WFT (*data not shown*) (i.e., this plant could serve as a source of this INSV vector). Organic as well as conventional growers interplant lettuce with strips of flowering annuals in order to attract beneficial insects. In the surveyed organic romaine field affected by INSV in this study, the interplanted sweet alyssum tested negative for INSV but supported large numbers of thrips, including some that tested positive for INSV. Thus, because sweet alyssum flowers are very attractive to thrips (hundreds of thrips were collected from the flowers), the results show how an apparent nonhost of INSV may play a role in the disease by serving as a source of the vector.

In summary, the IPM strategy for INSV in lettuce should involve planting virus- and thrips-free transplants, managing weeds and other sources of virus, monitoring and controlling thrips populations, avoiding the establishment of new plantings near lettuce fields or other crops with known INSV-infected plants or large populations of viruliferous thrips, and implementing prompt sanitation practices in lettuce fields following harvest. A long-term strategy should involve developing tospovirus-resistant lettuce varieties.

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