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Weedborne Reservoirs and Seed Transmission of *Verticillium dahliae* in Lettuce

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ABSTRACT

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The seed transmission of *Verticillium dahliae* was evaluated in lettuce (*Lactuca sativa*). Seed collected from lettuce plants infected with *V. dahliae* were plated with or without surface sterilization on Sorenson's modified NP10 medium. Of the seed plated with or without surface sterilization, 90 and 66%, respectively, yielded colonies of *V. dahliae*. The incidence of Verticillium wilt ranged from 55 to 80% among lettuce plants grown from seed harvested from infected plants. All evaluated isolates of *V. dahliae* were capable of seed transmission in lettuce. A *V. tricorpus* isolate failed to cause significant disease in lettuce or to become seedborne. Storage of contaminated seed at seven temperatures ranging from –20 to 15°C for up to 72 weeks did not reduce the incidence of *V. dahliae* in seed, whereas storage at room temperature (23 ± 2°C) for 20 to 52 weeks reduced the incidence of *V. dahliae* without affecting seed viability. Of the 11 weed species collected from fields with a known history of Verticillium wilt of lettuce, four yielded *V. dahliae*. Pathogenicity tests demonstrated that isolates of *V. dahliae* from *Sonchus oleraceus*, *Capsella bursa-pastoris,* and *Solanum sarrachoides* were as virulent as or more virulent than an isolate of *V. dahliae* from lettuce. These results demonstrate the potential of seedborne and weedborne inoculum to disseminate *V. dahliae*.

Verticillium wilt that occurs on many dicotyledonous plants is predominantly caused by the soilborne pathogens *Verticillium dahliae* Kleb. and *V. albo-atrum* Reinke & Berthold. Both pathogens colonize xylem tissues and cause disease on a broad array of plants (19). In some instances, these vascular pathogens may even invade the inflorescence and, subsequently, the developing fruit and seed (1,10,11,24). A third species, *V. tricorpus*, also is common to soil, but only considered a minor plant pathogen compared with *V. dahliae* and *V. albo-atrum* (19,28). All three fungi exhibit similar mycelium morphology. *V. dahliae* is distinguishable from *V. albo-atrum* by the presence of microsclerotia and the absence of highly melanized resting mycelia, whereas *V. albo-atrum* only produces melanized mycelia (28). *V. tricorpus* is distinguished by its capability to produce microsclerotia, melanized resting mycelia, and chlamydospores (28). The microsclerotia produced by *V. dahliae* serve as resting structures that are capable of surviving in soil under field conditions for up to 14 years in the absence of a host, making it a particularly difficult pathogen to control without the use of soil fumigants (36).

Wilts caused by *V. dahliae* have become a major problem in the cool-season production areas of coastal California over the past 10 years. *V. dahliae* is a prominent pathogen of a number of vegetable crops grown in the area (3,12), including those once considered nonhosts, such as lettuce (33). Few pathogens exhibit as diverse a host range as *V. dahliae* (14). Complicating this further is that many isolates of *V. dahliae* are also cross-pathogenic, capable of causing a range of symptom severities on several hosts (2,32). These individual isolates are usually most virulent on the host of origin. However, some are more host specialized and exhibit a limited host range (2). Cross-pathogenicity gives *V. dahliae* isolates the ability to persist within a field on hosts grown in rotation with lettuce and, potentially, on weed species susceptible to Verticillium wilt that are present within the production field or the surrounding noncultivated areas.

Verticillium wilt on lettuce (*L. sativa* L.) was identified in 1995 on a single ranch in coastal California and since has spread to other major lettuce production areas of this region (31). All market types of lettuce (i.e., head, leaf, romaine, and butter) are susceptible, although considerable variability exists in the degree of susceptibility among the lettuce types (33). A unique aspect of the disease on lettuce has been the association of an unusually high number of microsclerotia per gram of soil and wilt incidence. Nearly all fields that suffered extensive crop losses have contained between 200 and 2,500 microsclerotia g^{-1} of dry soil (31) in contrast to the range of 20 to 80 microsclerotia g^{-1} of dry soil described for a field continuously cropped with cotton for 7 years (20). Another unique feature of the lettuce crop itself and, consequently, of Verticillium wilt on lettuce is that lettuce is harvested in its vegetative stage when the disease also occurs. In most other crops, the disease is most severe when the crops are in the reproductive phase.

Although the modes of spread of *V. dahliae* are well defined in the literature (19), the unique aspects of the disease on lettuce require a reexamination of the pathogen transmission. Furthermore, even if the modes of transmission in lettuce are similar to those previously described in the literature, the duration of persistence of the seedborne inoculum has never been examined. The objectives of the present work were to (i) demonstrate the seedborne nature of *V. dahliae* in lettuce, (ii) assess the survival of *V. dahliae* in lettuce seed, and (iii) examine the potential of weed species common to lettuce production areas in central California to harbor isolates of *V. dahliae* pathogenic to lettuce.

MATERIALS AND METHODS

Lettuce plants and *Verticillium* isolates. Seed of the lettuce cv. Salinas and plant introduction (PI) 120938 were sown into autoclaved sand (1 h at 121°C) in 200-well seedling trays and maintained in the greenhouse (23 ± 5°C with ambient light) until needed. Isolates of *V. dahliae* from lettuce and artichoke (*Cynara scolymus* L.), and an isolate of *V. tricorpus* Isaac from artichoke (Table 1) were used to assess the seedborne nature of the pathogen in lettuce. Isolates were recovered (VdLs.17 in 1996 and the remaining isolates in 1998) from the roots of symptomatic plants in commercial fields in the Salinas and Pajaro Valleys, CA. Isolates were subcultured from single spores and stored on potato dextrose agar (PDA) slants at 4°C. The identities of isolates were confirmed based on key morphological characteristics (28).

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Determination of the seedborne nature of *V. dahliae* in lettuce. Inoculum for isolates of *V. dahliae* from artichoke and lettuce was prepared from 3-week-old cultures on PDA. Conidia were collected from each isolate and adjusted to a final concentration of 10^7 conidia ml^{-1} in sterile distilled water. Four-week-old seedlings of cv. Salinas were carefully removed and rinsed free of sand. Roots of the seedlings were trimmed 2 cm prior to dipping in suspensions of conidia for at least 5 min. Individual plants then were transplanted into 0.5-liter (16-oz.) insulated foam cups filled with autoclaved sand. For each isolate, at least 10 plants were inoculated, and at least 10 plants were dipped in sterile distilled water and maintained as uninoculated controls. Plants were arranged in a completely randomized design and maintained in the greenhouse for 8 weeks prior to evaluation for disease development. Vascular discoloration of plant roots then was scored by longitudinally cutting from the crown through the taproots to expose vascular tissues. The severity of vascular discoloration was recorded using a scale of 0 to 5, in which 0 = no vascular discoloration, 1 = 1 to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = 76 to 100% of vascular tissues exhibited discoloration in the absence of foliar symptoms; and 5 = 100% of vascular tissues exhibited discoloration and the wilting of foliar tissues typical of Verticillium wilt. The experiment was conducted twice. Analysis of variance was conducted to determine the overall effect of the *V. dahliae* isolates and *V. tricorpus* isolate on the severity of disease symptoms on lettuce. Replications within experiments were considered random effects in the analysis. The mean disease severity was calculated for each isolate and comparisons among isolates were made using Fisher's protected least significant difference test ($P \leq 0.05$).

Several of the uninoculated and inoculated plants from the above experiments were further maintained in the greenhouse to produce seed. Viable and nonviable seed and pericarp (made up of sclerenchyma and parenchyma) surrounding seed from inoculated and uninoculated plants were collected at maturity. Twenty-five seed were placed onto a modified Sorenson's NP10 semi-selective medium (9,30) either directly or following surface disinfestation with 70% ethyl alcohol for 20 s. Plates were incubated at room temperature (23 ± 2°C) and monitored for 3 weeks for seed germination (occurring generally within the first week) and the emergence of *V. dahliae* and other fungal colonies from viable and nonviable seed and pericarp from seed. The experiment was conducted twice. The percentage of viable seed, nonviable seed, and pericarp that yielded colonies of *V. dahliae* either from direct plating or plating after surface disinfestation was calculated for lettuce.

Experiments testing for seed transmission of *V. dahliae* to developing seedlings and the subsequent development of symptoms also relied on seed collected from lettuce plants inoculated with isolates of *V. dahliae* and *V. tricorpus,* as previously described. Seedlings were grown and maintained as previously described, except that 4-week-old seedlings were directly transplanted to 0.5-liter (16-oz.) insulated foam cups, keeping the roots and adhering soil intact. Plants were arranged in a completely randomized design and maintained in the greenhouse for 8 weeks prior to evaluation for disease development. Vascular discoloration of plant roots then was scored by longitudinally cutting from the crown through the taproots to expose vascular tissues. The percentage of plants that exhibited symptoms matching one of six disease classes was recorded, in which 0 = no vascular discoloration, 1 = 1 to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = 76 to 100% of vascular tissues exhibited discoloration in the absence of foliar symptoms; and 5 = 100% of vascular tissues exhibited discoloration and the wilting of foliar tissues typical of Verticillium wilt. The experiment was conducted twice.

Isolation of *V. dahliae* from weeds. The initial survey of weeds as potential carriers of *V. dahliae* was conducted in the ranch where the disease was first discovered (33). A number of weeds from the ditch banks (where a very high concentration of *V. dahliae* microsclerotia (2,500 g^{-1} of dry soil] was found) and around the fields were collected and transported to the laboratory. The roots and stems of weeds along with seed from inflorescences, if available (only in the case of two *Sonchus oleraceus* L. plants), were washed with tap water, surface disinfested in a solution of 70% ethyl alcohol for 20 s, and then thoroughly rinsed with sterile distilled water. Several excised pieces from surface-disinfested root and stem segments then were placed on a modified Sorenson's NP10 semiselective medium (9,30). Plates were incubated for at least 3 weeks at room temperature (23 ± 2°C) and periodically monitored for the presence of mycelia and microsclerotia typical of *V. dahliae*. The number of weeds yielding colonies of *V. dahliae* was recorded and the weeds were identified to the species level. Several resulting colonies of *V. dahliae* were further purified by single-spore isolations. Purified isolates were stored on PDA slants at 4°C prior to their use in lettuce pathogenicity experiments. The identities of all isolates were confirmed based on key morphological characteristics (28).

A systematic survey was conducted from April to October of 2001 and 2002. Weed species identified during the preliminary surveys, as well as other weed species, were arbitrarily collected from the production fields and noncultivated areas bordering fields where Verticillium wilt previously was observed in both Santa Cruz and Monterey Counties in California. These plants also were processed as described above and the number of weed species yielding colonies of *V. dahliae* was recorded and isolates purified and stored.

Pathogenicity of isolates of *V. dahliae* from weeds on lettuce. Isolates of *V. dahliae* recovered from *Capsella bursa-pastoris* (L.) Medik, *Senecio vulgaris* L., *Solanum sarrochoides* Sendtner, and *Sonchus oleraceus* L. were evaluated for pathogenicity on lettuce. Inoculum for each isolate was prepared as previously described and the density was adjusted to 10^7 conidia ml^{-1}. Four-week-old lettuce seedlings (cv. Salinas) were carefully removed and rinsed to remove sand from roots. Roots were trimmed 2 cm prior to dipping in suspensions of conidia from each isolate for at least 5 min. Individual plants then were transplanted into 0.5-liter (16-oz.) foam insulated cups filled with autoclaved sand. For each isolate, five plants were inoculated. Another five plants were dipped in sterile distilled water and maintained as uninoculated controls. Plants were arranged in a completely randomized design and maintained in the greenhouse for 8 weeks prior to uprooting and washing free of soil. Vascular discoloration of plant roots then was scored by longitudinally cutting from the crown through the taproots to expose vascular tissues. The severity of vascular discoloration was recorded using a scale of 0 to 5, in which 0 = no vascular discoloration, 1 = 1 to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = 76 to 100% of vascular tissues exhibited discoloration in the absence of foliar

Table 1. Severity of Verticillium wilt on lettuce plants (cv. Salinas) inoculated with isolates of *Verticillium dahliae* (Vd) from lettuce and artichoke, and an isolate of *V. tricorpus* (Vt)

Source of isolate	Isolate[y]	Disease severity[z]
Lettuce	VdLs.17	5.0 a
Artichoke	VdCs.208	4.0 a
Artichoke	VdCs.212	2.8 b
Artichoke	VtCs.225	0.3 c
…	Control	0.0 c

[y] Roots of 4-week-old seedlings were trimmed 2 cm and dipped into a 1×10^7 conidia ml^{-1} suspension for each isolate or dipped into sterile distilled water for the control, prior to transplanting. Plants were further maintained under greenhouse conditions for another 8 weeks.

[z] Mean disease severity of five plants was rated on a scale of 0 to 5, in which 0 = no vascular discoloration, 1 = 1 to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = 76 to 100% of vascular tissues exhibited discoloration in the absence of foliar symptoms; and 5 = 100% of vascular tissues exhibited discoloration and the wilting of foliar tissues typical of Verticillium wilt. Experiment was conducted twice with similar results. Values followed by the same letter are not significantly different based on the least significant difference test ($P = 0.05$).

symptoms; and 5 = 100% of vascular tissues exhibited discoloration and the wilting of foliar tissues typical of Verticillium wilt. The experiment was conducted twice. Analysis of variance was conducted to determine the effect of isolates from weeds on the severity of Verticillium wilt symptoms on lettuce. Replications within experiments were considered random effects in the analysis. The mean disease severity was calculated for each isolate of *V. dahliae* and comparisons among isolates were made using Fisher's protected least significant difference test ($P \leq 0.05$).

Effects of storage temperature on the viability of *V. dahliae* in seed. Two seed lots infested with *V. dahliae* were produced by inoculating 4-week-old lettuce seedlings of PI 120938 with isolate VdLs.17. The plants were maintained in the greenhouse until seed set. The seed was harvested, cleaned, and used to assess the effects of storage temperature on the survival of *V. dahliae* in infested seed. Random samples of infested seed were drawn from both seed lots, surface disinfested with a solution of 70% ethyl alcohol as previously described, and plated on a modified NP10 medium (9,30) to assess the degree of *V. dahliae* infestation. The plates were evaluated weekly for colonies of *V. dahliae* emerging from seed. Aliquots of seed (5 g) from each seed lot were placed in #2 coin envelopes and incubated at –20, –15, –10, 0, 5, 10, 15, and 23°C. Prior to incubating seed samples and every 2 to 4 weeks during the 72-week incubation, 21 seed from each incubation temperature of each seed lot were plated onto NP10 medium and monitored for germination and the emergence of *V. dahliae* and other fungi. The number of germinated seed and the number of seed yielding colonies of *V. dahliae* or other fungi was expressed as a percentage and plotted against time.

RESULTS

Virulence of *V. dahliae* isolates on lettuce. Lettuce plants (cv. Salinas) inoculated with isolates VdLs.17, VdCs.208, and VdCs.212 of *V. dahliae* exhibited mean disease severity ratings >2, whereas plants inoculated with the isolate of *V. tricorpus*, VtCs.225, exhibited a mean disease severity rating <2 (Table 1). No significant difference ($P \leq 0.05$) in mean disease severity ratings was observed between isolates VdLs.17 and VdCs.208, even though they originated from different hosts. However, isolates VdLs.17 and VdCs.208 were significantly ($P \leq 0.05$) more virulent than VdCs.212 from artichoke.

Seedborne nature of *V. dahliae* in lettuce. Of the viable seed harvested from plants inoculated with *V. dahliae*, 44 to 100% yielded *V. dahliae* (Table 2). *V. dahliae* also was recovered from 77 to 100% of the nonviable seed. No *Verticillium* sp. was detected on seed and pericarps from plants inoculated with the isolate of *V. tricorpus,* VtCs.225, or uninoculated control plants. Surface disinfestation of nonviable seed did not have a significant effect on the recovery of *V. dahliae*, but improved recovery from viable seed. Overall, surface disinfestation of either viable or nonviable seed, or pericarps, drastically reduced the recovery of other fungi.

Of the plants grown from seed infested with *V. dahliae*, 55 to 80% developed symptoms of Verticillium wilt (Table 3). No significant difference was observed in the transmission of *V. dahliae* among plants grown from those seed collected from apical versus axillary capitula (flower heads) of infected lettuce plants based on the incidence of Verticillium wilt (*data not shown*). Plants grown from seed harvested from uninoculated control plants and from seed of plants inoculated with *V. tricorpus,* VtCs.225, did not exhibit symptoms of Verticillium wilt.

Weed hosts of *V. dahliae*. *V. dahliae* was recovered from 4 of 11 weed species tested (Table 4). All root and stem tissues plated on modified NP10 medium (9,30)

Table 2. Recovery of *Verticillium dahliae* and other fungi from seed and floral tissues of infected lettuce (cv. Salinas) plants

			Percentage of seed contaminated[x]		
***Verticillium* isolate (source)[y]**	**Tissue/seed sampled**	**Surface sterilization (total samples)[z]**	**Verticillium**	**Other fungi**	**No fungi**
VdLs.17 (lettuce)	Viable seed	No (34)	100	29	0
		Yes (23)	100	0	0
	Nonviable seed	No (35)	100	43	0
		Yes (23)	100	0	0
	Pericarp	No (22)	68	95	0
		Yes (19)	74	26	21
VdCs.208 (artichoke)	Viable seed	No (27)	44	63	4
		Yes (23)	70	0	30
	Nonviable seed	No (31)	81	26	0
		Yes (26)	77	0	23
	Pericarp	No (23)	61	96	0
		Yes (23)	57	22	22
VdCs.212 (artichoke)	Viable seed	No (35)	54	66	0
		Yes (28)	100	0	0
	Nonviable seed	No (33)	100	27	0
		Yes (35)	86	3	11
	Pericarp	No (19)	58	100	0
		Yes (30)	47	50	3
VtCs.225 (artichoke)	Viable seed	No (26)	0	100	0
		Yes (26)	0	0	100
	Nonviable seed	No (29)	0	100	0
		Yes (25)	0	4	96
	Pericarp	No (23)	0	100	0
		Yes (28)	0	11	89
Control	Viable seed	No (23)	0	17	83
		Yes (21)	0	10	90
	Nonviable seed	No (21)	0	86	14
		Yes (21)	0	24	76
	Pericarp	No (21)	0	90	10
		Yes (21)	0	14	86

[x] Combined results from two experiments. Other fungi commonly observed on seed consisted mostly of common saprophytic species of *Aspergillus*, *Penicillium*, and *Cephlasporium*.

[y] Roots of 4-week-old seedlings were trimmed 2 cm and dipped into a 1×10^7 conidia ml^{-1} suspension for each isolate or dipped into sterile distilled water for the control, prior to transplanting. Plants were further maintained under greenhouse conditions until seed set. Vd = *V. dahliae* and Vt = *V. tricorpus*.

[z] Total number of sampled seed and tissues plated on a modified Sorenson's NP10 medium either with (yes) or without (no) surface sterilization using 70% ethyl alcohol.

Table 3. Incidence and severity of Verticillium wilt in lettuce plants (cv. Salinas) grown from seed infested with *Verticillium dahliae*

Verticillium isolate[x]	Germination (total seed)[y]	Disease incidence (%)[z]	Infected plants in each disease class (%)[w]					
			0	1	2	3	4	5
VdLs.17	57 (51)	80	21	14	3	14	10	38
VdCs.208	46 (24)	55	45	9	9	9	9	18
VdCs.212	43 (70)	63	37	10	7	0	27	20
VtCs.225	44 (39)	0	100	0	0	0	0	0
Control	78 (27)	0	100	0	0	0	0	0

[w] Percentage of plants in two greenhouse experiments that exhibited symptoms matching one of six disease classes, in which 0 = no vascular discoloration, 1 = 1 to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = 76 to 100% of vascular tissues exhibited discoloration in the absence of foliar symptoms; and 5 = 100% of vascular tissues exhibited discoloration and the wilting of foliar tissues typical of Verticillium wilt. Values were rounded to the nearest whole number and may not total 100%.

[x] Lettuce seed infested with individual *V. dahliae* (Vd) isolates or a *V. tricorpus* (Vt) isolate were collected from greenhouse-grown plants that originally were inoculated as 4-week-old seedlings. Roots of seedlings were trimmed 2 cm and dipped into a 1×10^7 conidia ml^{-1} suspension for each isolate or dipped into sterile distilled water for the control, prior to transplanting.

[y] Percentage of total seed from plants that germinated in two greenhouse experiments.

[z] Percentage of plants in two greenhouse experiments that exhibited root discoloration typical of Verticillium wilt.

Table 4. Isolation of *Verticillium dahliae* from weeds collected from lettuce production areas in central California with a history of Verticillium wilt

Botanical name	Common name	Plants tested	*V. dahliae*-infested (%)[z]
Capsella bursa-pastoris (L.) Medik	Shepherd's purse	4	100
Solanum sarrachoides Sendtner	Hairy nightshade	2	100
Sonchus oleraceus L.	Sowthistle	27	32
Senecio vulgaris L.	Groundsel	5	20
Raphanus sativus L.	Wild radish	2	0
Amaranthus hybridus L.	Pigweed	2	0
Chenopodium album L.	Lambsquarters	1	0
Conyza canadensis (L.) Cronq.	Mares tail	1	0
Xanthium spinosum L.	Spiny clotbur	1	0
Polygonum lapathifolium L.	Curlytop knotweed	1	0
Lactuca serriola L.	Wild lettuce	1	0

[z] Percentage of plants sampled from which *V. dahliae* was recovered when placed on a modified Sorenson's NP10 medium.

Table 5. Severity of Verticillium wilt on lettuce plants (cv. Salinas) inoculated with several isolates of *Verticillium dahliae* collected from weeds

Source of isolate	Isolate[y]	Disease severity[z]
Groundsel	VdSv.449	0.4 c
Hairy nightshade	VdSs.494	5.0 a
Hairy nightshade	VdSs.495	4.0 a
Lettuce	VdLs.17	2.6 b
Shepherd's purse	VdCb.486	0.4 c
Shepherd's purse	VdCb.487	2.2 b
Sowthistle	VdSo.428	2.2 b
Sowthistle	VdSo.451	0.4 c
Sowthistle	VdSo.452	0.4 c
Sowthistle	VdSo.493	0.6 c
Sowthistle	VdSo.503	0.0 c
Sowthistle	VdSo.504	0.2 c
…	Control	0.0 c

[y] Roots of 4-week-old seedlings were trimmed 2 cm and dipped into a 1×10^7 conidia ml^{-1} suspension for each isolate or dipped into sterile distilled water for the control, prior to transplanting. Plants were further maintained under greenhouse conditions for another 8 weeks.

[z] Mean disease severity of 5 plants rated using a scale of 0 to 5, in which 0 = no vascular discoloration, 1 = 1 to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = 76 to 100% of vascular tissues exhibited discoloration in the absence of foliar symptoms; and 5 = 100% of vascular tissues exhibited discoloration and the wilting of foliar tissues typical of Verticillium wilt. Experiment was repeated twice with similar results. Values followed by the same letter are not significantly different based on the least significant difference test ($P = 0.05$).

yielded *V. dahliae*. Of the 11 weed species sampled from fields or areas bordering those fields with a history of Verticillium wilt of lettuce, sowthistle was the most predominant. About 32% of the sampled sowthistle plants yielded colonies of *V. dahliae*. Seed collected from the inflorescences of two sowthistle plants also yielded colonies of *V. dahliae*. The other 10 species of weeds were not as numerically represented as sowthistle, reflecting their relative abundance during sampling, but many still yielded colonies of *V. dahliae*.

Pathogenicity of *V. dahliae* isolates from weeds on lettuce. Of the 11 isolates of *V. dahliae* tested from various weeds, 3 isolates (VdSo.428, VdCb.487, and VdSs.495) were as virulent as the representative isolate from lettuce, VdLs.17, and one isolate (VdSs.494) was more virulent than VdLs.17 (Table 5). Lettuce plants inoculated with the remaining seven isolates exhibited mean disease severity ratings <2 and were considered nonpathogenic on lettuce.

Effects of storage temperature on the viability of *V. dahliae* in seed. At the onset of the experiment, the two seed lots composed of seed naturally infested with *V. dahliae* did not significantly differ with respect to seed germination (*t* test; $P = 0.68$). Seed sampled from lot 1 exhibited a lower incidence of *V. dahliae* (*t* test; $P \leq 0.001$) and a higher incidence of other fungi (*t* test; $P \leq 0.001$) compared with seed sampled from lot 2. However, the effect of the different storage temperatures over time on the survival of *V. dahliae* in seed was similar for both seed lots (Figs. 1 and 2). Seed germination within each seed lot remained relatively unchanged until week 56, when the germination for seed stored at ambient room temperature (23°C) began to decline in both seed lots. Recovery of *V. dahliae* also showed a decline after 16 to 20 weeks of storage at 23°C in both seed lots. At all other temperatures, the recovery of *V. dahliae* from seed remained high, from 10 to 71% and 57 to 100% for lots 1 and 2, respectively. Data collection for both seed lots stored at 15°C was discontinued after 40 weeks, when the seed was inadvertently exposed to water while in storage.

Recovery of fungi other than *V. dahliae* varied little for either seed lot over the 68 to 72 weeks of storage across the different temperatures, except in seed stored at 23°C (Figs. 1 and 2). In lot 1, there was a noticeable increase in the incidence of other fungi (non-*Verticillium* spp.) in seed stored at 23°C compared with seed stored at other temperatures from 20 to 52 weeks. A similar increase in the incidence of other fungi occurred in seed from lot 2 stored at 23°C from 48 to 68 weeks. In both instances, the increase in other fungi corresponded with the decrease in the percentage of seed contaminated with *V. dahliae*.

DISCUSSION

The seedborne nature of *V. dahliae* has been documented in cotton (1), eggplant, tomato (10), and spinach (29), and in the cultivated composites safflower and sunflower (19,24,26,37). We report the seedborne nature of *V. dahliae* in lettuce under greenhouse conditions. All isolates of *V. dahliae* inoculated to lettuce were capable of seed transmission, regardless of the

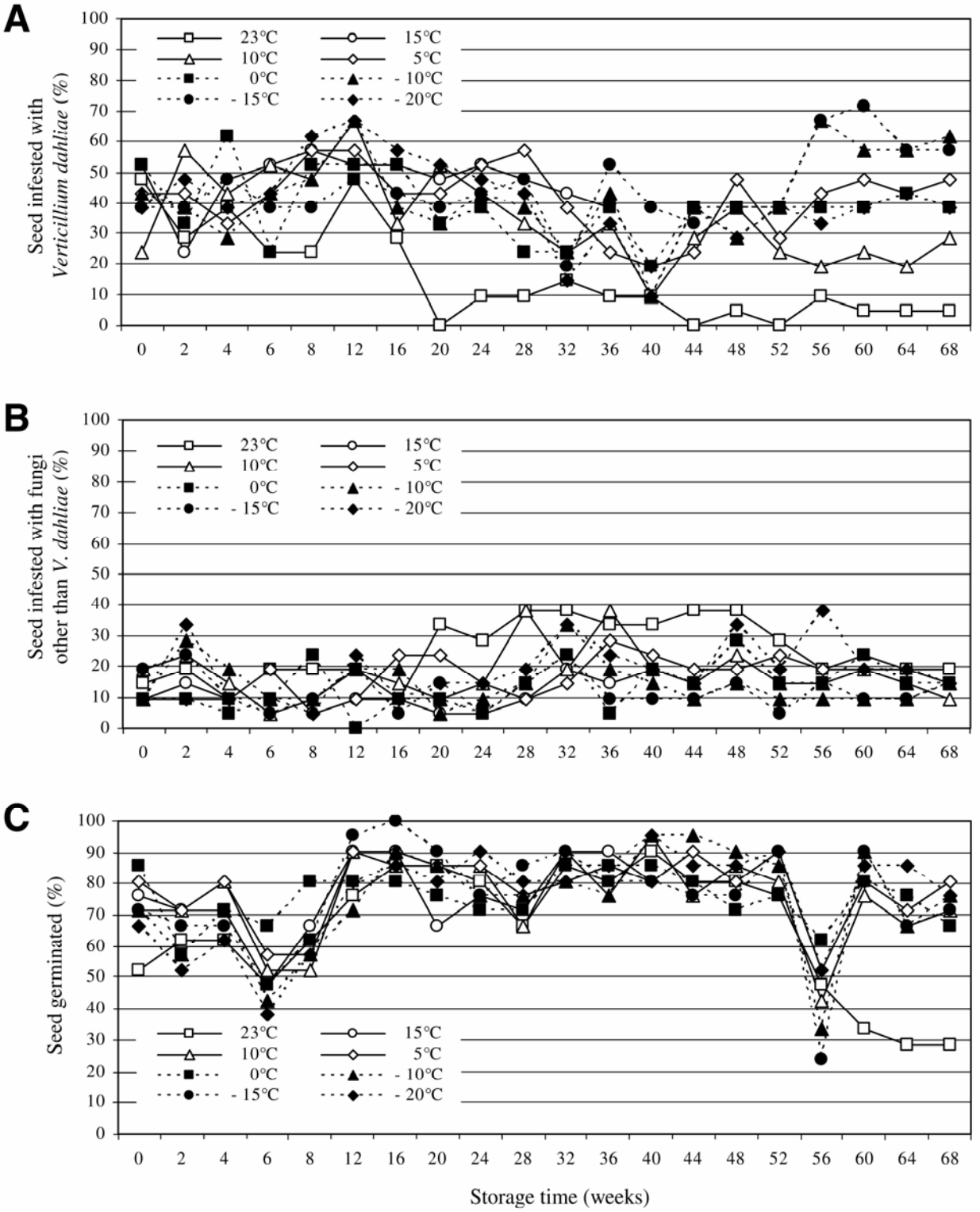

Fig. 1. Effect of storage temperature on the recovery of **A,** *Verticillium dahliae* and **B,** other fungi from seed, and **C,** seed germination in seed lot 1. Over a 68-week period, the percentage of seed (n = 21) sampled from seed lot 1 that exhibited colonies of *V. dahliae* and other fungi and germination was determined by plating seed onto a modified Sorenson's NP10 medium. Seed storage at 23°C was performed at ambient room temperature (±2°C), whereas other storage temperatures were performed in controlled incubators. Data collection for seed stored at 15°C was discontinued after 40 weeks due to an accidental exposure of the seed to water while in storage.

relative virulence of the isolate or whether the isolate originated from lettuce or artichoke. The minor wilt pathogen, *V. tricorpus* (VtCs.225), failed to inflict significant disease symptoms on lettuce or to contaminate seed in inoculated plants (19). This is the first report of seed transmission of *V. dahliae* in *L. sativa*, which previously was reported to be a new host of *V. dahliae* (15,33).

The recovery of *V. dahliae* from infested seed and floral tissues was aided by surface disinfestation, most likely by reducing the presence of competing fungi on the seed or tissue surface. The recovery of *V. dahliae* following the disinfestation of seed surfaces suggests that the fungus resides within the achene, similar to findings in safflower and sunflower (11,24). In preliminary studies, the pericarps shed from germinating lettuce seed were colo-

nized by *V. dahliae,* although whether any internal tissues are colonized remains unknown (G. E. Vallad and K. V. Subbarao, *unpublished data*). The shed pericarp from a germinating lettuce seed generally remains near the developing root system and could contain sufficient inoculum to facilitate infection of the developing plants. In safflower and sunflower, where *V. dahliae* was confined to the pericarp and outer surface of the testa of infected seed, the pathogen never entered the testa (11,24).

Our studies determined that *V. dahliae* can survive in lettuce seed for prolonged periods of time, especially when seed is stored at temperatures below 15°C. Other researchers also have reported on the survival of *Verticillium* spp. in infested seed over extended durations. Sackston and Martens (24) noted that *V. dahliae* survived in naturally infested sunflower seed for 16

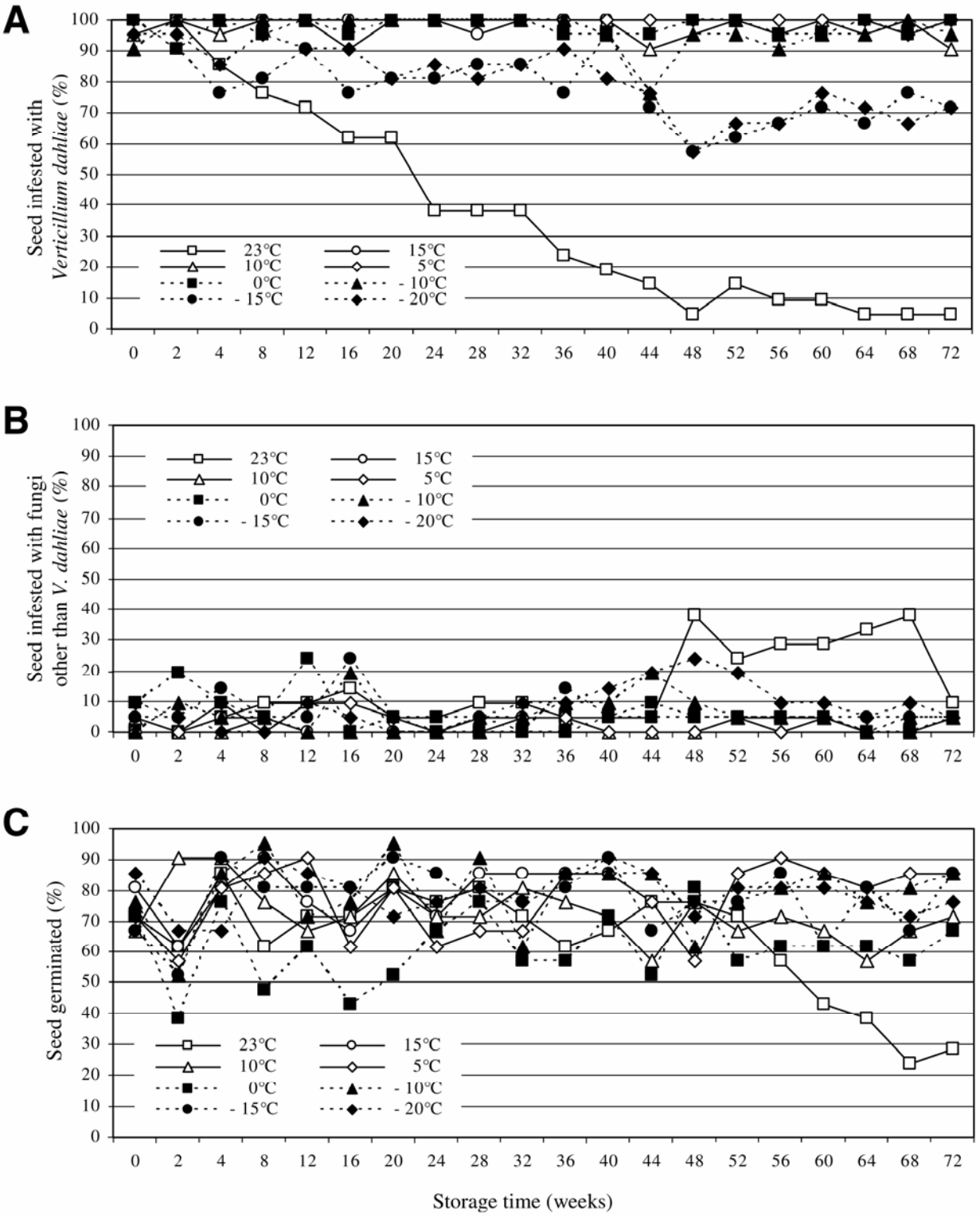

Fig. 2. Effect of storage temperature on the recovery of **A,** *Verticillium dahliae* and **B,** other fungi, and **C,** seed germination in seed lot 2. Over a 72-week period, the percentage of sampled seed (n = 21) from seed lot 2 that exhibited colonies of *V. dahliae* and other fungi and germination was determined by plating seed onto a modified Sorenson's NP10 medium. Seed storage at 23°C was performed at ambient room temperature (±2°C), whereas other storage temperatures were performed in controlled incubators. Data collection for seed stored at 15°C was discontinued after 40 weeks due to an accidental exposure of the seed to water while in storage.

months of storage at ambient conditions in an unheated facility in Manitoba, Canada. Allen (1) reported the survival of *V. albo-atrum* in artificially infected seed of cotton for up to 8 months. Klisiewicz (11) found that the fungus remained viable in safflower seed for at least 2 years.

Prolonged storage at 15 to –20°C did not reduce the seedborne incidence of *V. dahliae* in lettuce, but storage at room temperature (23°C) for 20 weeks or longer did. However, there also was a corresponding decline in the viability of seed stored at room temperature. The drop in seed viability is not conclusive, because germination tests were not performed in a typical manner on germination paper, but rather on the same modified NP10 medium used to detect *V. dahliae*. However, it has been our experience that viable lettuce seed generally germinates within a week of being placed on the modified NP10 medium (G. E. Vallad and K. V. Subbarao, *unpublished data*).

Few studies have been reported for the effect of storage temperature on the survival of *V. dahliae* in seed and other plant materials. The prolonged storage of strawberry runner plants for 18 to 34 days at 1°C was reported to reduce the recovery of *V. dahliae* in infected plants and the incidence of Verticillium wilt in the field following transplanting (27). Some success at eliminating *V. dahliae* from infested eggplant seed by treating with hot water at 49°C also has been reported (10). Future experiments should test the effect of higher storage temperatures and hot-water treatments on the survival of *V. dahliae* in lettuce seed.

Regardless of the virulence or host of origin, seed transmission of *V. dahliae* to seedlings of *L. sativa* leading to disease occurred under greenhouse conditions. The importance of seedborne inoculum in the dissemination of *V. dahliae* has been debated in the literature (10,21,22,34). Even though seed transmission of *V. dahliae* was demonstrated in cotton (24), eggplant, and tomato (10), the work of Rudolph (21) and Rudolph and Harrison (22), while acknowledging that seed transmission was possible, argued that it was "improbable and of no economic significance" in California production due to the low incidence of disease they observed in controlled field trials. This debate over the importance of seedborne inoculum stems from the erratic nature of seed infestation and the differences in seed transmission observed in greenhouse and field studies, often attributed to variability in the environment (10,11,23–25). The biological implications of our studies need to be tested further under field conditions.

Although we have not directly tested for seed transmission of *V. dahliae* under field conditions, we were able to recover *V. dahliae* from seed of several symptomatic lettuce plants recovered from the field (*data not shown*). These lettuce plants were uprooted at harvest maturity from a field, transplanted to pots, and then maintained in a greenhouse for another 30 days until seed harvest. Seed from these plants yielded *V. dahliae* colonies when plated on NP10 medium. In the lettuce seed production areas of the San Joaquin Valley, CA and elsewhere, seed is threshed directly from the bolted plants in the field (33) and these seed have not been tested for *V. dahliae* recovery.

A preliminary survey suggested that several common weed species harbored *V. dahliae*. These weeds were collected from a field with a known history of Verticillium wilt of lettuce. Of the weeds collected that harbored *V. dahliae*, all were identified previously as symptomatic or asymptomatic carriers of *V. dahliae* (6,7,15,19), with the exception of *Solanum sarrachoides* Sendtner (hairy nightshade). We not only observed foliar or root symptoms common to Verticillium wilt among plants of *S. sarrachoides* and *Sonchus oleraceus* (*data not shown*) but also isolated *V. dahliae* from seed of *S. oleraceus* (*data not shown*). Evans (6) concluded that *S. oleraceus* was a nonhost of *V. dahliae* because the infection was locally confined to plant roots that would often harbor fungal counts similar to susceptible hosts, but lacked the production of microsclerotia. Several reports state that both *Capsella bursa-pastoris* (L.) Medik and *Senecio vulgaris* are hosts of *V. dahliae*, but vary in regards to the extent of internal colonization and external symptoms (4,8,35). Environmental and genotypic variation among geographic locations and host–pathogen interactions, respectively, likely explain the differences observed by previous researchers.

Further studies are required to determine the extent of *V. dahliae* among weed species in California. All of the weeds surveyed in this study that failed to recover *V. dahliae* were classified as susceptible by other researchers, except *Polygonum lapathifolium* L. (curlytop knotweed) and *Conyza canadensis* (L.) Cronq. (mare's tail) (4,6,8,15,19,35). However, several other species of *Polygonum*, including *P. pennsylvanicum* L., *P. convolvulus* L., and *P. persicaria* L., previously were classified as hosts of *V. dahliae* (6–8). Several monocots, although generally regarded as nonhosts of *V. dahliae*, also have been reported to exhibit disease symptoms and to harbor localized populations of *V. dahliae* capable of forming microsclerotia (8,13,14,16–18). Sixty other members of the family *Asteraceae* (*Compositae*) and 22 other members of the family *Solanaceae* are listed as known hosts of *V. dahliae* or *V. albo-atrum* (19); therefore, the addition of *Solanum sarrachoides* to the growing list of hosts is not surprising.

The susceptibility of several weed species to *V. dahliae* and the infestation of seed and subsequent infection of seedlings from these seed has the potential to spread the pathogen in coastal California. *L. serriola* (prickly lettuce) is broadly distributed throughout California and is a major weed commonly associated with lettuce production in the Salinas and Pajaro Valleys of central California. Although we did not sample any *L. serriola* plants during our surveys, several greenhouse experiments have demonstrated the susceptibility of *L. serriola* to lettuce isolates of *V. dahliae* (data not shown), stressing the importance of controlling these weeds in problematic growing areas, even when the afflicted fields are in rotation with another crop (like artichokes). The susceptibility of *Lactuca* spp. to isolates of *V. dahliae* from lettuce, in addition to the susceptibility of lettuce to several isolates of *V. dahliae* that were collected from weed species (including an isolate that was more virulent than the isolate from lettuce), raises concerns about the potential of weed species to act as a reservoir of *V. dahliae* in California vegetable production areas. Lettuce was not considered a host of *V. dahliae* until 1995, when a surprising outbreak of Verticillium wilt was observed in three lettuce fields totaling 20 ha in Watsonville, CA (33). Since 1995, Verticillium wilt has spread to 200 ha throughout the Salinas and Pajaro Valleys in Monterey and Santa Cruz Counties, CA. In greenhouse experiments, an isolate of *V. dahliae* from lettuce was pathogenic on nearly every vegetable produced in the area (2), severely limiting the practical use of crop rotations to control this disease. Now the additional finding of several weed species capable of harboring the pathogen adds an additional layer of complexity to this disease. Several other studies have shown that weed species are an important source of *V. dahliae* often neglected in epidemiological studies (4,6,8,15,35). Evans (5,6) found that several weed species, including *Xanthium pungens* L. and *X. spinosum* L. often harbored *V. dahliae* isolates pathogenic to cotton. More importantly, these *Xanthium* spp. could produce seedborne inoculum that Evans (6) hypothesized could explain the association between the spread of weed hosts and the spread of Verticillium wilt of cotton throughout the Namoi Valley of New South Wales, Australia (5,6). Our studies demonstrate that similar factors, seed transmission and weed hosts, are in place in the Salinas Valley and could be partially responsible for the spread of *V. dahliae* throughout the area, in addition to other mechanisms of spread induced by wind and various human activities. Future research should clarify the roles of weedborne and seedborne inoculum in the epidemiology of Verticillium wilt under field conditions.

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