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Irrigation Scheduling Impacts Vegetative Growth, Seed Yield, and Fungal Diseases of Spinach Seed Crops in a Maritime Mediterranean Climate

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Abstract. **Although irrigation scheduling has been studied for diverse vegetable crops, much less attention has been given to irrigation scheduling for the seed crops on which these production systems rely. In spinach, for which irrigation scheduling needs are likely to vary greatly between seed and leaf production, this leaves seed producers without adequate resources to make irrigation scheduling decisions. Our research sought to fill this gap by evaluating two alternative irrigation scheduling strategies (a publicly available decision-support tool and soil moisture sensors) and four soil moisture thresholds for irrigation for their impacts on vegetative growth, marketable seed yield, seed quality, and the severity of Stemphylium leaf spot (caused by *Stemphylium vesicarium* and *Stemphylium beticola*), a common foliar disease of spinach, under sprinkler irrigation. We found that in all 3 years of the study, earlier and more frequent irrigation increased vegetative growth. However, marketable seed yield only increased relative to the control treatment based on farmers' standard irrigation practices in 1 of the 3 years—a year with an abnormally late planting date. This indicates that vegetative growth is more responsive than seed yield to earlier and more frequent irrigation, and that increases in vegetative growth do not translate directly to increased marketable seed yield. Contrary to the expected increase in Stemphylium leaf spot severity with increasing irrigation, the severity decreased in both years it was measured, likely as a result of the small stature of the spinach seed parent lines used in our study and opportunistic pathogenicity on moisture-stressed plants. These results provide a useful foundation from which spinach seed producers can make irrigation management decisions for their crops that underpin a valuable global industry.**

Changing precipitation patterns and competition for water resources have spurred considerable research on irrigation management and alternative irrigation strategies in vegetable crops (e.g., De Pascale et al. 2011; Singh et al. 2019; Zinkernagel et al. 2020). Although this research is critical to sustaining more than 1.1 billion mt of global vegetable production (Food and Agriculture Organization of the United Nations 2021), there has been little research on irrigation practices for the vegetable seed crops on which this vegetable production relies. The limited research that has been conducted on irrigation for vegetable seed crops has focused on the relationship between the timing and severity of moisture stress and seed yield, seed quality, and/or seed vigor (seed size, seed weight, radicle length, and percent germination), particularly for lettuce and carrot seed production. For example, Izzeldin et al. (1980) investigated the relationship between soil matric potential values of –30, –80, and –500 kPa held constant, punctuated once in an otherwise constant moisture regime, or cycled through the vegetative and reproductive phases in lettuce seed production in a greenhouse. They found that the highest yielding treatments were those held at –80 kPa (the "moderate" irrigation deficit treatment) either through the vegetative phase only or throughout both phases. In contrast, treatments in which the –500 kPa threshold was reached sometime during the reproductive growth phase (and to a lesser extent if only in the vegetative phase) were the lowest yielding, but had the largest seeds and the greatest seed vigor as determined by seed weight, size, and density. Similarly, in a greenhouse study, Contreras et al. (2008) found that restricting irrigation at bolting reduced lettuce plant dry weight and seed yield per plant, but increased seed weight, germination, and radicle length. In carrot seed crops grown in the field on a sandy loam soil, Steiner et al. (1990) found that daily irrigation to replace evapotranspiration (ET) produced greater seed yield than less frequent irrigation, but in the treatment that received less frequent irrigation, irrigation quantities of 60% to 120% of ET produced a similar seed yield, and a reduction in seed yield was not observed until 40% of ET. The authors also found that seed yield was reduced if irrigation (at 100% of ET) was stopped 5 or 7 weeks before harvest compared with if irrigation was stopped 2 weeks before harvest. Although these results suggest that carrot and lettuce seed crops are sensitive to moisture stress during the reproductive phase, it is unknown how these results translate to other vegetable seed crops.

Leaf spinach is produced on over 22,000 ha in the United States and is valued at almost $500 million US (US Department of Agriculture, National Agricultural Statistics Service 2021). A substantial portion of this production is baby leaf spinach, which requires dense planting and high seeding rates. However, the cultivation of spinach seed for leaf spinach production is very limited geographically, requiring areas with average summer temperatures $< 28\,^{\circ}C$ (Navazio and Colley 2007) and daylengths in excess of 16 h, which are needed to initiate bolting in most modern varieties (Chun et al. 2001). For example, up to 10% of global spinach seed production takes place in just two counties in Washington State, USA, as a result of the restricted climatic needs of this crop (Foss and Jones 2005). Research on irrigation in leaf spinach production has generally shown that leaf spinach is extremely sensitive to moisture stress, and that even minor water deficits as low as 80% of daily ET (Ekinci et al. 2015; Leskovar and Piccinni 2005) or soil matric potential values as high as field capacity (Nasarullah et al. 2022; Seymen 2021) can decrease fresh and dry plant biomass, leaf area and number, and properties such as chlorophyll and protein content in both the greenhouse (Ekinci et al. 2015; Nasarullah et al. 2022; Seymen 2021) and the field (Leskovar and Piccinni 2005). In contrast, Zhang et al. (2014) found that a mild deficit of 20% to 30% of field capacity actually increased yield compared with full replacement in a field trial on a silt loam soil, as did Nishihara et al. (2001) in a sandy soil in a greenhouse study, although both studies showed that this yield benefit disappeared quickly as water deficits increased. Whether the sensitivity of leaf spinach yield to moisture stress translates to the yield of spinach seed crops remains to be seen.

In addition to the direct impact of irrigation on yield, free moisture or high relative humidity in the plant canopy can affect the incidence and severity of fungal plant pathogens

(Koike et al. 2001; Manda et al. 2021). *Stemphylium* spp., including *Stemphylium vesicarium* and *Stemphylium beticola*, are important fungal pathogens on crops globally, including spinach (Koike et al. 2001), garlic (Basallote-Ureba et al. 1999), lentil (Mwakutuya and Banniza 2010), onion, pear, rye, and asparagus (Foster et al. 2019). Of particular concern is *S. vesicarium*, which is rapidly developing resistance to dicarboximides, strobilurins, and other fungicide classes (Alberoni et al. 2006; Hay et al. 2019), threatening the production of pear and onion in addition to spinach. The fungal disease caused by these *Stemphylium* spp. in spinach, Stemphylium leaf spot (SLS), is especially problematic for fresh-market spinach production because it creates a papery lesion that damages the marketability of the leaf itself (Koike et al. 2001). However, it is also a known disease of spinach seed crops (du Toit and Derie 2001; du Toit et al. 2005, 2006). Previous research suggested that *Stemphylium botryosum* was the primary causal agent of SLS in spinach (du Toit and Derie 2001; Koike et al. 2001; Reed et al. 2010), but recent analyses by Liu et al. (2020) and Spawton et al. (2019) found that what was believed to be *S. botryosum* was more closely related to *S. beticola* based on recent changes in the taxonomy of the genus (Woudenberg et al. 2017).

Given the importance of spinach seed crops as the foundation of global spinach production, the dearth of research on irrigation management for spinach seed crops, and the potential for consequential interactions between irrigation management and major fungal diseases, we investigated two alternative irrigation scheduling strategies and several soil moisture thresholds for irrigation to determine their impact on seed yield, vegetative biomass, canopy cover, harvest index, plant nutrient status, seed quality, seed health, and foliar SLS severity. We hypothesized that earlier and more frequent irrigation would increase marketable seed yield and vegetative biomass, but that it would also increase SLS incidence and severity.

Materials and Methods

Study site description and field management. This study was conducted from 2019 to 2021 at the Washington State University Northwestern Washington Research and Extension Center in Mount Vernon, WA, USA (lat. 48°26'21.4"N, long. 122°23'12.5"W). Spinach seed crops are typically planted on a 10- to 15-year rotation because of the potential for crop loss caused by *Fusarium oxysporum* f. sp. *spinaciae*, and, as such, the study rotated between adjacent fields each year. The 2019 and 2020 trials were conducted on a Skagit silt loam—fine-silty, mixed, superactive, nonacid, mesic Fluvaquentic Endoaquepts (National Cooperative Soil Survey 2005)—with a pH of 6.5 in 2019 and a pH of 6.7 in 2020, and 2.6% organic matter. The available water capacity (AWC) of the soil is ~0.20 $cm \cdot cm^{-1}$, according to Web Soil Survey (Soil Survey Staff 2023). The 2021 trial was conducted on a Sedrowoolley silt loam—a coarse-silty, isotic, nonacid, mesic Vitrandic Xerofluvents (National Cooperative Soil Survey 2002)—with a pH of 6.1 and 2.9% organic matter. The AWC of the soil is ~0.19 $cm \cdot cm^{-1}$ (Soil Survey Staff 2023). Preseason soil fertility at the research trial locations based on a composite soil sample sent to a commercial soil test laboratory can be found in Supplemental Table 1. The region has a Mediterranean climate with 80 cm of precipitation annually, but only 26.3 cm of precipitation from April to September (Fig. 1). The average daily high temperature is 14.7 °C, which typically peaks in July or August (AgWeatherNet 2020).

In general, field maintenance followed recommended practices for spinach seed crops in this region. Spinach parent lines were proprietary and provided by partner seed companies, and the parent lines were different each year. Planting density was ~173,000 plants/ha, with 66 cm between rows and a 7.6-cm plant spacing within rows in 2019 and 2021. In 2020, the planting density was estimated at 253,000 plants/ha, with the same spacing between rows and an ~6-cm plant spacing within rows. The higher seeding rate was chosen by the collaborating seed company because of the later planting date in 2020 (details described later). A 17–7–7 8.5S–3.9Mg–0.2B fertilizer blend was broadcast and incorporated at 485.7 $kg \cdot ha^{-1}$ on 10 Apr 2019, an 18–18–6 7.8S–3.5Mg–0.2B blend was applied at a rate of 534.2 $kg \cdot ha^{-1}$ on 24 Apr 2020, and a 19–23–4 5.7S–2.3Mg blend was applied at a rate of 645.6 $kg \cdot ha^{-1}$ on 21 Apr 2021. In 2021, lime was applied at a rate of 4483.4 $kg \cdot ha^{-1}$ on 13 Apr. A cycloate herbicide (Ro-Neet®; Helm Crop Solutions, Tampa, FL, USA) at 3 $L \cdot ha^{-1}$ was preplant incorporated on 22 Apr 2019, on 1 May 2020, and on 10 May 2021. A bifenthrin insecticide (Capture® LFR™; FMC, Philadelphia, PA, USA) at 0.5 $L \cdot ha^{-1}$ was applied with the cycloate herbicide (Ro-Neet®) in 2019 and 2020, and the bifenthrin insecticide Bifender® FC (Vive Crop Protection, Mississauga, Ontario, Canada) was applied in 2021 for insect pest control. An azoxystrobin fungicide (Aframe™; Syngenta, Greensboro, NC, USA) at 1.1 $L \cdot ha^{-1}$ also was applied on 1 May 2020. Planting occurred 24 Apr 2019, 1 May 2020, and 12 May 2021. In 2020, the field was replanted on 2 Jun because of poor emergence. A methyl sulfanilylcarbamate herbicide (Asulox®; UPL Ltd, Warrington, Cheshire, UK) at 3.5 $L \cdot ha^{-1}$ was applied on 25 May 2019, 23 Jun and 6 Jul in 2020, and 4 and 16 Jun in 2021. Between-row cultivation was conducted once or twice

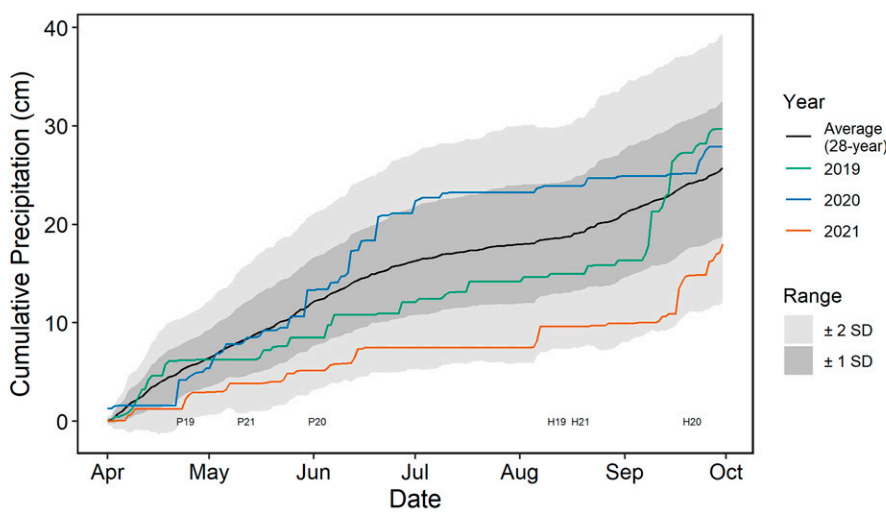

Fig. 1. Cumulative precipitation received during the growing season (1 Apr–30 Sep) in Mount Vernon, WA, USA, in 2019 (green), 2020 (blue), and 2021 (orange) compared with the 28-year mean (black). One and two standard deviations (*SD*) from the 28-year average are shown in dark and light gray, respectively. Planting and harvest dates are indicated by P or H, respectively, and the year. Data were taken from current (for 2021) and emeritus (for 1994–2020) station data recorded by Washington State University's AgWeatherNet (2020).

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each year by collaborating farmers and, in 2021, pelletized 46–0–0 urea was applied by a collaborating farmer during cultivation on 22 Jun.

Experimental design and irrigation treatments. The experiment was arranged in a randomized complete block design with four (in 2019) or five (in 2020 and 2021) replicates. The main plots were 4.0 × 9.1 m, with 15.2 (in 2019) or 6.1 m (in 2020 and 2021) of buffer between within-replicate plots and 8.2 m between replicates. Each main plot was planted with six rows of spinach, 0.66 m apart, where the center four rows were female plants and the outer two rows were male plants. Irrigation treatments were applied to main plots, and in 2020 and 2021, plots were further subdivided into two 3.7- × 4.0-m split plots, with a 1.8-m buffer between, that were or were not inoculated with the causal agent of SLS (described in the next section).

In each of the 3 years (2019–21), two irrigation scheduling strategies, soil moisture thresholds (as determined by soil matric potential sensors) and a decision support tool based on a calculated water balance called Irrigation Scheduler (IS) were compared with a control treatment that received no irrigation (NI) and a control treatment that was irrigated based off the activity of a nearby reference farmer with the same spinach parent line, a similar planting date, and a similar soil series [the farmer's control (FC)]. No parameterization or calibration was performed for the IS treatment to mimic the most likely usage of the decision support tool by farmers. In 2019, the soil moisture sensor treatment was irrigated whenever the soil matric potential dropped to –110 kPa, whereas in 2020 and 2021, variable soil moisture thresholds for sensor-based scheduling were also included. Thus, in 2020 and 2021 there were a total of seven treatments: four treatments irrigated whenever the soil matric potential reached –75, –100, –125, or –150 kPa (SM75, SM100, SM125, and SM150, respectively), the two controls (NI and FC), and the treatment irrigated based on the IS decision support tool available from Washington State University (Peters et al. 2019). Soil moisture thresholds were chosen to span the range of soil matric potentials typically observed in spinach seed production fields in the region.

In 2019, treatments were irrigated with mobile aluminum hand lines and a single adjustable brass impact sprinkler (model 25PJDA-C; Rain Bird®, Azusa, CA, USA) at the center of each plot to simulate irrigation with a hose reel traveling impact sprinkler, which is the most common irrigation method in the region. The distribution uniformity (DU) of this system was determined to be unacceptably low at 56%, so in 2020 and 2021, all irrigated plots were equipped with six microsprinklers (model MP2000-90 Rotator®; Hunter Industries Inc., San Marcos, CA, USA) at 60-cm-high arranged in a square grid with 5.5-m spacing. These sprinklers were chosen to simulate irrigation with hose reel traveling boom cart sprinklers, an increasingly prevalent system in the region, and the DU for this new system was determined to be 76%. In all years, plots were irrigated to an equivalent depth of 2.5 cm in all applicable treatments except IS treatment plots, which were irrigated to replenish the projected water deficit whenever it exceeded 1.3 cm. Soil matric potential was monitored three times per week in all treatments using granular matrix sensors (WATERMARK® 200SS; Irrometer Company, Inc, Riverside, CA, USA), which were installed after planting at the center of the plot in the southernmost female plant row. Sensors were installed at a depth of 23 cm, the midpoint of the expected root zone based on the work of Schenk et al. (1991). For all threshold treatments, irrigation occurred when the average soil matric potential of all replicates within that treatment fell below the irrigation threshold. Average and minimum soil matric potential readings ± the standard error (*SE*) in kilo-Pascals by irrigation treatment for the vegetative and reproductive growth stages of the spinach seed crop can be found in Supplemental Table 2.

Inoculation treatments for SLS. To evaluate the potential impact of irrigation management on SLS, a common foliar disease of spinach crops, main plots were divided into inoculated and uninoculated split plots in 2020 and 2021. Isolates of *S. beticola* and *S. vesicarium* were first isolated from leaves of local spinach seed crops 1 to 3 years earlier. One isolate of *S. beticola* was used in the 2020 trial and one isolate each of *S. beticola* and *S. vesicarium* were used in the 2021 trial. Isolates of each species were grown out on eight petri plates (two plates per isolate) of clarified V8 (CV8) agar medium [200 mL V8 Original® (Campbell Soup Company, Camden, NJ, USA), 4.5 $CaCO_3$, 800 mL deionized water, and 15 g Bacto™ granulated agar (Becton, Dickinson, and Company, Sparks, MD, USA), centrifuged before water was added at 3500 rpm for 15 min] and incubated at room temperature for 2 weeks. Isolates were then transferred to 100 daughter petri plates (in 2020, 25 per isolate) or 200 daughter petri plates (in 2021, 50 per isolate) of CV8 agar medium in the form of colonized plugs from the mother plates and were incubated at room temperature for 2 weeks.

Inoculum was prepared by blending isolate plates with water in a blender and straining the agar with fine cheesecloth (in 2020) or by scraping sporulation from plate surfaces and blending with water without the addition of the agar (in 2021). The volume of inoculum required was calculated by using a target application rate of 9.35 $L\cdot ha^{-1}$ and a backpack sprayer volume output of 242 $mL\cdot 15\ s^{-1}$ for a final target volume of 23 L. Deionized water was added to the inoculum until this volume was achieved. Final propagule counts were performed using a hemocytometer and were as follows, expressed as the average of two counts per inoculation: 1.71×10^5 (first inoculation) and 2.36×10^5 (second inoculation) propagules/mL for consecutive inoculations in 2020, and 3.06×10^4 and 3.00×10^4 propagules/mL for consecutive inoculations in 2021. In 2020, these propagules consisted entirely of mycelial fragments because of the sparse sporulation of the fungal cultures. A surfactant (TWEEN® 80; Croda International PLC, Snaith, UK) was added at a rate of 0.1 $mL\cdot L^{-1}$ immediately before spraying.

Inoculant was applied at anthesis using a four-nozzle backpack sprayer equipped with flat fan nozzles (model TeeJet® 8003 TeeJet Technologies, Glendale Heights, IL, USA) and coarse strainers. Sprays were done at sunset without leaf wetting in 2020 and with 3 min of leaf wetting using installed microsprinklers to facilitate infection more effectively in 2021 (including the nonirrigated control).

Canopy cover. To assess the impact of irrigation management on plant growth, canopy cover was measured five times in 2020 (every other week: 22 Jun, 7 and 20 Jul, and 4 and 18 Aug) and twice in 2021 (approximately monthly: 8 Jun and 9 Jul). Three 66-× 66-cm quadrats were established in fixed locations in each plot throughout the season. Images were taken so they were completely parallel to the soil surface and encompassed the entire quadrat, and were subsequently cropped to include only the quadrat. The images were processed using the web application Canopeo v. 2.0 (Oklahoma State University, Stillwater, OK, USA) to determine the percentage of canopy cover (Patrignani and Ochsner 2015).

Leaf tissue nutrient status. For leaf tissue analysis (in 2020 and 2021 only), 30 recently matured leaves per plot were collected from female plant rows in a distributed, grid-based manner to capture a representative sample of the whole plot. Leaves were collected once per season (7 Jul 2020 and 6 Jun 2021), when female stem elongation had initiated. In 2020, samples were collected only from plots with the most and least irrigated treatments (NI and IS); and in 2021, leaves were sampled from plots of all treatments. Samples were dried at 55.7 °C for 4 d, crushed and ground, and sent to a commercial soil and plant tissue analysis laboratory (Soiltest Farm Consultants, Inc, Moses Lake, WA, USA) for analysis of total N, P, K, Ca, Mg, S, Zn, Fe, Mn, Cu, and B.

Foliar disease incidence and severity. The incidence and severity of SLS (causal agent: *S. beticola* or *S. vesicarium*) was assessed in 2020 and 2021, and the incidence of spinach downy mildew disease (downy mildew; causal agent: *Peronospora effusa*) was assessed in 2021 only. For SLS, two ratings per year were performed by a consistent team of three raters at 2 and 4 weeks after inoculation (7 and 14 Aug 2020, and 16 and 30 Jul 2021) in the female plant rows. Split plots (inoculated and uninoculated) were rated separately, and ratings were performed block-by-block. In 2020, overall severity of SLS in each subplot was rated on a 1- to 5-point scale because the severity was too low to estimate reliably the percentage of leaf area affected. In 2021, percentage leaf coverage was estimated and given a rating of 0% to 100%. There was incidence of SLS in all plots in both years, including in uninoculated subplots, and as such, only the severity of SLS was considered further.

The incidence of downy mildew was assessed by randomly sampling lower leaves from eight randomly selected plants within each plot. Visible sporulation on the undersides of leaves was noted to give percent incidence.

Vegetative biomass and seed yield. Swathing and harvest took place on 12 Aug 2019 and on two dates each in 2020 and 2021 (10 and 21 Sep 2020, 10 and 19 Aug 2021). Harvest data were taken from 3-m sections of the two central female rows in each of the inoculated and uninoculated split plots. Two 3-m sections were also taken from the northernmost male row of each plot, one from each split plot in 2020 and from the uninoculated split plot in 2021 only. Samples were field-dried for 1 to 3 d and then dried to a constant weight at 60 °C for male plants and 27 to 32 °C (to preserve seed viability) for female plants. Total aboveground biomass was measured for male plants, which were then discarded. The total aboveground biomass was also measured for female plants, which were then run through a plot thresher (Model LPT-MRB, ALMACO, Nevada, IA, USA) three times in 2019 and 2021, and two times in 2020. Seeds were cleaned using a seed separator (Clipper© M2B; A.T. Ferrell Company Inc, Bluffton, IN, USA) and manual single-belt separator, then a tabletop separator (Clipper©) was used to isolate marketable seed sizes (screen sizes 7–13). Threshed seed was dried at 27 to 32 °C to a constant weight and then the total seed weight was subtracted from the total aboveground biomass to obtain the vegetative biomass. The harvest index was calculated by dividing the marketable seed yield by the total aboveground biomass to determine the effect of irrigation quantity and frequency on seed production efficiency.

Seed quality and seed health. To evaluate the effects of irrigation management on seed quality and health, thousand seed weight counts, and germination and disease transmission assays were performed. For thousand seed counts, a random subsample of 1000 seeds from each plot and split plot were dried at 30 °C for 48 h before being weighed. For germination, two replicates of 50 seeds from each plot were placed onto moist germination paper. The paper was incubated in a plastic bag at room temperature for 3 weeks, with percent normal germination recorded at 7, 14, and 21 d; and percent abnormal germination, percent decayed seeds, and the percentage of seeds that did not germinate recorded at 21 d. As an index of seed vigor, the mean length of incubation time (MLIT) was calculated according to Edwards (1934),

$$\text{MLIT} = \frac{\sum_i^c T_i N_i}{\sum_i^c N_i},$$

where T_i is the percentage of seeds that germinated at day i, N_i is the number of days since the beginning of the germination test, and c is the number of days counts were made in the germination test.

To assess both *Stemphylium* spp. and *Verticillium dahliae* incidence on seed, we followed the International Seed Health Initiative's NP-10 agar medium method (International Seed Health Initiative 2017). Agar medium was prepared by combining one autoclaved mixture containing 5 g SIGMA-grade polygalacturonic acid (Na from orange), 1 g NaOH pellets (0.025 N), and 500 mL water with another autoclaved mixture containing 15 g Bacto™ agar, 1 g each KNO_3 and H_2PO_4, 0.5 g each KCl and $MgSO_4 \cdot 7H_2O$, 0.5 mL Tergitol NP-10® (Dow Chemical Company, Midland, MI, USA) and 500 mL distilled water. After each autoclaved mixture was cooled to 50 °C, 0.5 g each of the dry antibiotics chloramphenicol, streptomycin sulfate, and chlortetracycline hydrochloride were added. Agar medium was cooled to 50 °C after autoclaving and was poured into 10- × 10-cm lidded acrylic boxes. Seed was surface-sterilized by stirring with 1.2% NaOCl solution in strainers for 1 min followed by three 30-s stirrings in distilled water. One hundred seeds per plot (inoculated subplots only; 500 seeds per treatment) were plated in the agar boxes and incubated on a diurnal light cycle at 24 °C for 9 d. Seeds were inspected with a dissecting microscope (Leica® model Mz125; Leica Microsystems Inc, Deerfield, IL, USA) at 5 and 9 d. Presence or absence of the following were noted: *Stemphylium* conidia, *Stemphylium* pseudothecia, *Verticillium* conidia, and *Verticillium* microsclerotia.

Data analysis. A linear mixed-effect model was developed using RStudio software (R Core Team 2022) v. 4.1.0 (2021-05-18, "Camp Pontanezen") and v. 4.2.2 (2022-10-31, "Innocent and Trusting"), and the "nlme" package (Pinheiro et al. 2023). Irrigation treatments, inoculation treatments (nested within irrigation treatments when applicable), and year were treated as fixed effects whereas block was included as a random effect. Interactions among all three fixed effects were included. Assumptions of homoskedasticity and normality were checked first by plotting residuals, then were confirmed with the Levene and Shapiro-Wilk tests. Tukey's honestly significant difference test was used for separation of the treatment means. For tests when data were recorded over multiple dates (canopy cover, seed health, seed germination), only the final date was analyzed, as this date either yielded the most reliable data or the date did not change the interpretation of the data. For tests when subsamples were used to increase accuracy (canopy cover), subsamples were averaged before analysis of variance.

Results

Irrigation and soil moisture. The number of irrigation events per treatment in a single season ranged from 0 (NI) to 12 (IS), and the amount of water applied per treatment in a single season ranged from 0 (NI) to 25.5 cm (SM75) (Table 1). In 2019 and 2020, plots with the driest soil moisture threshold treatments (–110 kPa and –150 kPa, respectively) received less water than plots with the FC treatment. However, in 2021, when only 5.6 cm of precipitation occurred between planting and harvest compared with 6.1 cm in 2019 and 18.3 cm in 2020, and there were 49 consecutive days with no rain, this trend was reversed. Similarly, although the number of irrigation events and the amount of irrigation applied in the two wetter soil moisture threshold treatments (SM75 and SM100) and the IS treatment changed relatively little between 2020 and 2021, irrigation in the driest threshold treatments (SM125 and SM150) increased markedly in quantity and frequency, with more than three times as much water applied in the SM125 and SM150 treatments in 2021 as in 2020 (Table 1).

Soil matric potential values showed a clear response to irrigation and precipitation (Fig. 2), although given that the fields typically received more precipitation during the vegetative growth phase, differences in the average soil matric potential between irrigation treatments were less pronounced in this phase than in the reproductive phase (Supplemental Table 2). Trends in soil matric potential were similar across years, with less frequent rainfall as the season progressed resulting in declining soil moisture in the treatment plots that received little or no irrigation. Despite less cumulative precipitation received during the growing season in 2019 than in 2020, the absence of long intervals between rain events in 2019 maintained greater soil moisture levels in the NI treatment than in either 2020 or 2021.

Canopy cover. There was a significant year-by-treatment interaction for canopy cover, as shown in Table 2, and as such, a post hoc

Table 1. Irrigation frequency and amount of irrigation applied for irrigation scheduling treatments in a spinach seed crop grown in Mount Vernon, WA, USA in 2019 to 2021.

	Irrigation applied (cm) by year			No. of events by year		
Treatment[i]	2019	2020	2021	2019	2020	2021
NI	0	0	0	0	0	0
FC	4.8	6.6	4.1	3	2	2
IS	14.7	20.1	18.1	8	12	10
SM75	—	25.5	22.8	—	8	8
SM100	—	14.8	19.7	—	6	7
SM110	2.1	—	—	1	—	—
SM125	—	6.1	19.7	—	2	7
SM150	—	3.1	11.1	—	1	4

[i] Treatments were no irrigation (NI), a farmer's control (FC) based on the practices of the nearest farmer with the same parent line and a similar planting date and soil type, irrigation based on the decision support tool Irrigation Scheduler (IS) from Washington State University's AgWeatherNet, and four soil matric potential thresholds for irrigation based on soil moisture sensors: –75 (SM75), –100 or –110 (SM100 or SM110, respectively), –125 (SM125), and –150 kPa (SM150). Approximately 2.5 cm of irrigation was applied during each irrigation event for all treatments, with the exception of the IS treatment, for which the decision support tool dictated the amount applied as long as it was at least 1.25 cm.

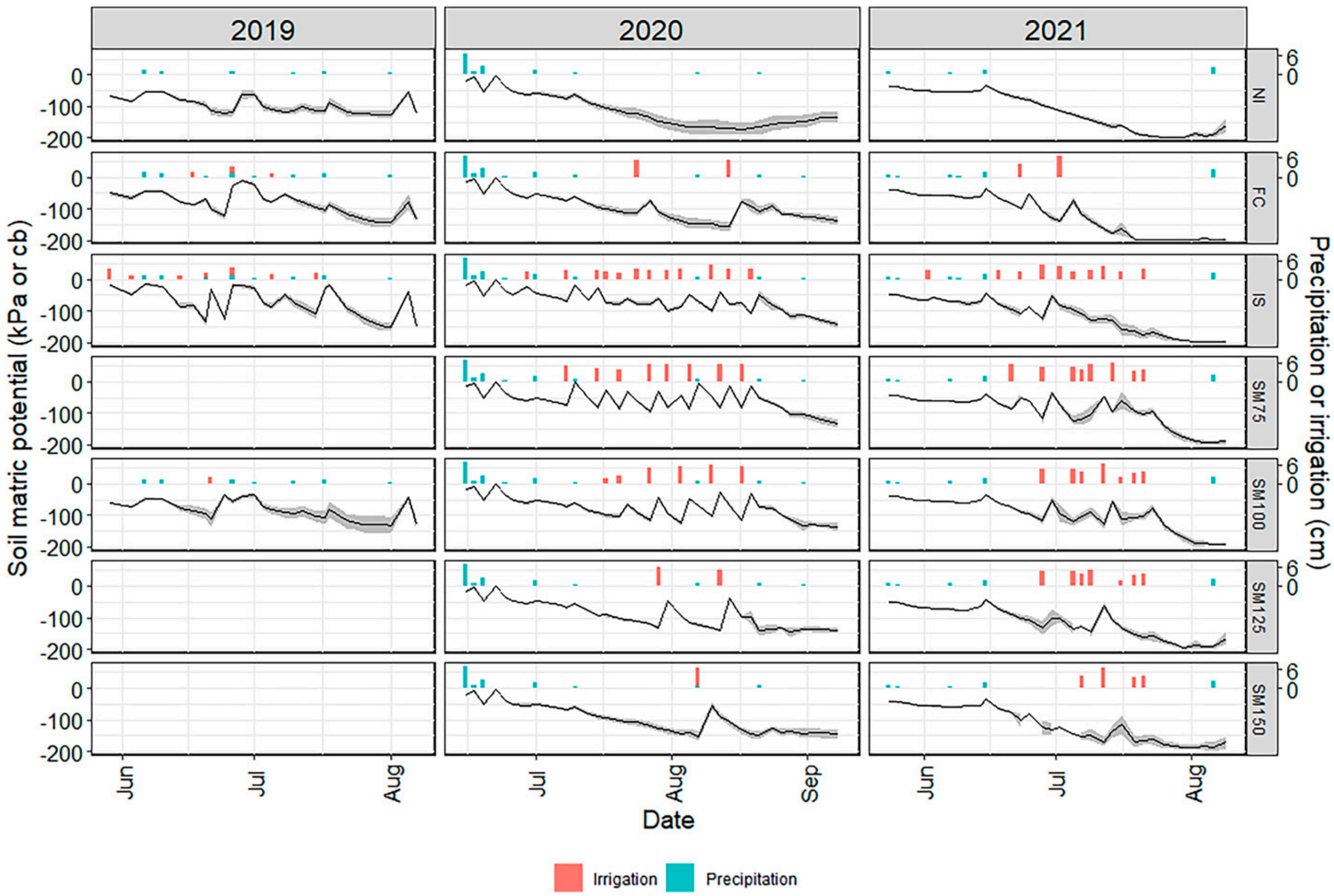

Fig. 2. Average soil matric potential (gray shading shows ± standard error) by treatment and the amount of precipitation and irrigation for all 3 years of the study. Treatments were no irrigation (NI), a farmer's control (FC) based on the practices of the nearest farmer with the same parent line and a similar planting date and soil type, irrigation based on the decision support tool Irrigation Scheduler (IS) from Washington State University's AgWeatherNet, and four soil matric potential thresholds for irrigation based on soil moisture sensors: –75 (SM75), –100 (SM100), –125 (SM125), and –150 kPa (SM150). Note that in 2019 the threshold for irrigation in the SM100 treatment was actually –110 kPa but it is represented here as SM100 for ease of comparison. Soil matric potential was measured three times per week with granular matrix sensors (Watermark 200SS; Irrometer Company Inc, Riverside, CA, USA) and precipitation data are from Washington State University's AgWeatherNet (2020). The transition from vegetative to reproductive growth (bolting) occurred on ∼21 Jun 2019, 13 Jul 2021, and 21 Jun 2021.

means separation was conducted separately for each year. Irrigation treatments impacted spinach canopy cover ($P < 0.001$ in both 2020 and 2021), with earlier and more frequent irrigation increasing percent coverage significantly (Table 3). Percent canopy cover increased by 160% from the NI treatment to the –75-kPa threshold treatment in 2020 and by 60% in 2021. Mean percent canopy cover was 28% higher across all treatments in 2021 than in 2020, with the greatest increases in the drier treatments (e.g., a 77% increase in the NI treatment vs. an increase of only 6% in the IS treatment). Inoculation treatments did not impact canopy cover (Table 4).

Table 2. *P* values for linear mixed-effects modeling of the treatment effects and interactions on plant response variables.

Effect[i]	Canopy cover	Vegetative biomass, female[ii]	Vegetative biomass, male	Seed yield	Harvest index[ii]	Germination percentage	Thousand seed wt	MLIT[iii]	Abnormal sprouts	Decayed seeds	Nongerminated seeds
Year	**<0.001**[iv]	**0.002**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**0.006**	**<0.001**	**<0.001**
Treatment[v]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.058	**<0.001**	0.320
Inoculation	0.165	**0.004**	—	**0.030**	0.300	**0.012**	**0.017**	0.073	0.129	**0.003**	**0.020**
Year × treatment	**0.003**	0.634	**0.002**	**<0.001**	**0.007**	**<0.001**	**0.004**	**0.004**	0.388	**<0.001**	0.684
Year × inoculation	0.346	—	—	**0.004**	—	0.092	0.119	**0.004**	0.982	**0.027**	0.946
Treatment × inoculation	0.821	0.569	—	0.815	0.493	0.281	0.666	0.063	0.397	0.276	0.535
Year × treatment × inoculation	0.988	—	—	0.180	—	0.413	**0.006**	0.278	0.983	0.131	0.682

[i] Data are from 2020 and 2021 only. Data from 2019 were analyzed separately for all variables because of the different treatment structure in 2019 (only a subset of irrigation treatments and no inoculation treatments).

[ii] Biomass data were not collected from inoculated females in 2020. Inoculation effects include data from 2021 only.

[iii] Mean length of incubation time (MLIT) was calculated as the product of the number of days since the start of the germination assay at each time point and the percentage of germinated seeds at that time point, summed for each time point measured, and divided by the sum of the percentage of germinated seeds at each time point.

[iv] *P* values in bold type are statistically separable at $P < 0.05$.

[v] Irrigation treatments applied to a spinach seed crop in Mount Vernon, WA, USA. Treatments were no irrigation, a farmer's control based on the practices of the nearest farmer with the same parent line and a similar planting data and soil type, irrigation based on the decision support tool Irrigation Scheduler from Washington State University's AgWeatherNet, and four soil water potential thresholds for irrigation based on soil moisture sensors: –75, –100 or –110, –125, and –150 kPa.

Table 3. Impact of irrigation scheduling treatments on plant growth and seed yield.[i]

Treatment[ii]	Canopy cover (%)	Vegetative biomass (kg·ha^{-1}) Female[iii]	Male	Marketable seed yield (kg·ha^{-1})	Harvest index[iii]
2019					
NI	—	1948 ± 263	3349 ± 471	2506 ± 236	0.56 ± 0.01 a
FC	—	2115 ± 399	3495 ± 483	2459 ± 272	0.55 ± 0.03 ab
IS	—	3560 ± 663	5012 ± 1207	2587 ± 219	0.43 ± 0.04 b
SM110	—	2524 ± 721	4919 ± 555	2793 ± 651	0.54 ± 0.02 ab
P value	—	0.204	0.364	0.919	**0.035**
2020					
NI	23.04 ± 2.05 b	4528 ± 576 b	3073 ± 286 c	1930 ± 192 d	0.44 ± 0.01
FC	35.96 ± 2.15 ab	5889 ± 355 ab	3488 ± 440 bc	2604 ± 118 cd	0.46 ± 0.01
IS	55.84 ± 2.84 a	8298 ± 791 a	5621 ± 746 ab	3540 ± 137 ab	0.47 ± 0.05
SM75	59.92 ± 4.37 a	8322 ± 1112 a	5747 ± 510 a	3602 ± 284 a	0.41 ± 0.02
SM100	51.03 ± 4.04 ab	6797 ± 494 ab	4117 ± 568 a–c	2850 ± 157 bc	0.44 ± 0.02
SM125	36.09 ± 2.76 ab	5904 ± 376 ab	3644 ± 237 a–c	2521 ± 107 cd	0.41 ± 0.02
SM150	27.82 ± 2.48 b	4600 ± 434 b	2495 ± 405 c	2043 ± 90 d	0.43 ± 0.01
P value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.473
2021					
NI	40.77 ± 1.84 c	1457 ± 85 c	976 ± 153 b	1742 ± 127 c	0.52 ± 0.01 a
FC	50.02 ± 2.68 bc	2265 ± 226 bc	1505 ± 161 ab	2503 ± 38 ab	0.50 ± 0.01 a
IS	58.95 ± 2.79 ab	3065 ± 241 ab	1754 ± 247 ab	2892 ± 129 a	0.48 ± 0.02 a
SM75	65.07 ± 2.66 a	3691 ± 276 a	1846 ± 263 a	2363 ± 87 a–c	0.37 ± 0.02 c
SM100	59.92 ± 2.51 ab	3038 ± 233 ab	1890 ± 151 a	2391 ± 182 a–c	0.42 ± 0.01 bc
SM125	54.09 ± 2.10 a–c	3103 ± 253 ab	1702 ± 148 ab	2472 ± 166 ab	0.43 ± 0.01 b
SM150	41.49 ± 1.42 c	1906 ± 281 c	1415 ± 147 ab	2195 ± 181 bc	0.48 ± 0.01 a
P value	**<0.001**	**<0.001**	**0.025**	**0.001**	**<0.001**

[i] Values shown are the mean ± standard error. When *P* values are less than 0.05 (indicated in bold type in the table), values followed by the same lowercase letter are not statistically separable based on Tukey's honestly significant difference.

[ii] Irrigation treatments applied to a spinach seed crop in Mount Vernon, WA, USA. Treatments were no irrigation (NI), a farmer's control (FC) based on the practices of the nearest farmer with the same parent line and a similar planting date and soil type, irrigation based on the decision support tool Irrigation Scheduler from Washington State University's Ag Weather Net, and four soil water potential thresholds for irrigation based on soil moisture sensors: –75 (SM75), –100 or –110 (SM100 or SM110, respectively), –125 (SM125), and –150 kPa (SM150).

[iii] Only uninoculated plants are included in 2020.

Vegetative biomass. Vegetative biomass showed similar trends to canopy cover, increasing with earlier and more frequent irrigation in both male and female parent lines (Table 3), although the trend in 2019 did not meet $\alpha < 0.05$ for either the male or female parent lines. In 2020, the SM75 treatment increased female vegetative biomass by 84% over the NI and SM150 treatments, and increased male biomass by 130% over the NI, FC, and SM150 treatments. In 2021, the SM75 treatment (for females) and the SM75 and SM100 treatments (for males) increased biomass by 153% over the NI, FC, and SM150 treatments (for females), and by 94% over the NI treatment (for males). There was an overall average increase in vegetative biomass of 98% between the plants in the treatment with the lowest vegetative biomass (mostly NI) and the plants in the treatment with the highest vegetative biomass (mostly IS or SM75) across males and females in all years. It should be noted that in 2020, vegetative biomass data were only available for plants that were not inoculated with *S. beticola* and *S. vesicarium*. Overall, there was less vegetative spinach biomass in the 2021 trial compared with the 2020 trial, likely as a result of differences in parent lines, planting density, and the severity of SLS. Inoculation with *S. beticola* and *S. vesicarium* decreased vegetative biomass by 17% in 2021, the only year in which data for both inoculated and uninoculated plants were available (Table 4).

Seed yield. Marketable seed yield increased with earlier and more frequent irrigation in both 2020 and 2021 ($P < 0.001$ in 2020 and $P = 0.001$ in 2021), although there was greater separation among the treatments in 2020 (Table 3). In 2020, plots treated with four of the irrigation treatments (SM125, SM150, FC, and NI) yielded less seed than plots with the two most irrigated treatments (IS and SM75); in 2021, only the SM150 and NI treatments had a lower seed yield than the most irrigated treatment (IS). In 2020, there was an 87% increase in seed yield between the lowest yielding treatment (NI) and the highest yielding treatment (SM75). In 2021 there was a 66% increase in seed yield between the lowest yielding treatment (NI) and the highest yielding treatment (IS). Seed yield was also impacted by the inoculation treatments, with a 19% decrease in seed yield from the uninoculated treatment to the inoculated treatment across 2020 and 2021 ($P = 0.030$; Table 4). The harvest index decreased with increasing irrigation in 2019 and 2021 ($P = 0.035$ and $P < 0.001$, respectively), given the increases in vegetative biomass observed with increasing irrigation, but the absent (in 2019) or relatively lower (in 2021) impacts of irrigation on marketable seed yield compared with vegetative biomass (Table 3). In 2020, strong impacts of irrigation on both vegetative biomass and seed yield resulted in no impact of irrigation on the harvest index ($P = 0.473$). Irrigation water productivity (measured as kilograms of seed per cubic meter of water applied) generally increased with decreasing irrigation ($P < 0.001$ in all years) and was maximized in whichever treatment received the least irrigation in each year (Supplemental Table 3).

Seed quality. No relationship between irrigation treatments and percent germination was observed in 2019 or 2020, although in 2021, more frequent irrigation (IS, SM75, SM100) resulted in higher germination than with the FC and NI treatments ($P < 0.001$; Table 5). There was a 23% increase in percent germination from the NI treatment (with the lowest germination) to the IS treatment (with the highest germination). There was a corresponding decrease in the percentage of abnormal sprouts and the percentage of decayed seeds with increasing irrigation in 2021 ($P = 0.043$ and $P < 0.001$, respectively), but no difference in the percentage of abnormal sprouts in other years, and no clear relationship between irrigation and the percentage of decayed seed in other years. A similar pattern for seed weight emerged, in which there was no statistical relationship between irrigation and thousand seed weight in 2019 or 2020; but, in 2021, there was a small but detectable relationship ($P < 0.001$). Unlike in the germination assays, the trend was for lower seed weight with more irrigation, apart from the IS treatment, which had the highest seed weight and was comparable to SM150 and NI. There was an 11% increase in thousand seed weight from the lowest weight treatment (SM75) to the highest weight treatment (IS). The speed of germination, as measured by the MLIT, differed among

Table 4. P values for linear mixed-effects modeling of the inoculation treatment effects on plant response variables.[i]

Treatment	Canopy cover (%)	Female vegetative biomass (kg·ha^{-1})[ii]	Marketable seed yield (kg·ha^{-1})	Harvest index[ii]	Germination percentage	Thousand seed wt	MLIT (d)[iii]	Abnormal sprouts percentage	Decayed seeds percentage	Nongerminated seeds percentage
Inoculated	43.60 ± 4.07	2738 ± 224	2123 ± 141	0.44 ± 0.01	73.11 ± 2.31	11.98 ± 0.22	8.90 ± 0.10	9.62 ± 0.40	12.76 ± 0.96	4.51 ± 0.38
Noninoculated	48.91 ± 3.51	3315 ± 299	2608 ± 141	0.45 ± 0.02	76.74 ± 2.19	12.38 ± 0.24	8.75 ± 0.11	8.77 ± 0.41	10.84 ± 0.87	3.65 ± 0.38
P value	0.165	**0.004**[iv]	**0.030**	0.300	**0.012**	**0.017**	0.320	0.138	0.138	0.114

[i] Includes data from 2020 and 2021 only.

[ii] Values are from 2021 only.

[iii] MLIT = mean length of incubation time.

[iv] Values in bold type are statistically separable at $P < 0.05$.

irrigation treatments in both 2020 and 2021, and more irrigation generally led to slower germination times ($P \leq 0.001$) in addition to smaller seed size. Seeds from the NI treatment germinated 1.4 and 0.6 d faster on average in 2020 and 2021, respectively, than seed from the IS treatment. Both germination and thousand seed weight were impacted slightly by the inoculation treatments, with overall decreases between the uninoculated and inoculated treatments of 5% and 3%, respectively ($P = 0.012$ and $P = 0.017$, respectively; Table 4).

Leaf tissue nutrient status. In 2020, only Mn and Fe differed between the most (IS) and least (NI) irrigated treatments (the only two treatments tested), with Mn decreasing by 49% and Fe increasing by 51% with increasing irrigation ($P = 0.02$ and $P = 0.03$, respectively; Table 6). In 2021, total N, Ca, and Mg all increased with increasing irrigation (by 26%, 29%, and 37%, respectively, between the NI and IS treatments; $P < 0.001$ for each), whereas Fe showed the opposite trend from the prior year and decreased by 16% from the NI to the IS treatment ($P = 0.011$).

Stemphylium leaf spot severity. Given that there was an interaction between the irrigation and inoculation treatments in 2021 ($P = 0.003$; Table 7), the results are separated by inoculation treatment. The results are also separated by year because of the different rating scales used in each year as a result of the differing SLS severity. Treatment plots that received the least irrigation had the greatest disease severity in both years ratings were assigned, regardless of whether the subplot was inoculated with *S. beticola* and *S. vesicarium* or not (Table 8). In 2020, SLS severity increased by 123% in the inoculated plots and by 160% in the uninoculated plots from the treatment with the lowest severity (IS) to the treatment with the highest severity (FC; $P = 0.006$ and $P < 0.001$, respectively; Table 8). In 2021 the trend was similar, with an increase of 120% in the inoculated plots and 331% in the uninoculated plots from the treatment with the lowest severity (SM100) to the treatment with the highest severity (NI; $P = 0.001$ and $P < 0.001$, respectively).

Downy mildew incidence. Downy mildew incidence showed the opposite trend to SLS severity in 2021 (the only year downy mildew was observed and its incidence was recorded), with more frequent irrigation and greater irrigation amounts resulting in a higher incidence of downy mildew ($P = 0.017$; Table 8). In the treatment with the highest downy mildew incidence (SM100), 50% of leaves were affected compared with only 7.5% in the FC treatment, a greater than 6-fold increase.

Seed health assays. There was no relationship between irrigation treatments and the presence of morphological structures identifiable as *Stemphylium* spp. in our seed health assays ($P = 0.223$; Table 8). The seed health assay was only conducted in 2021 and only on seed from the subplots inoculated with *S. beticola*

Table 5. Impact of irrigation scheduling treatments on germination and seed vigor indices in spinach seeds.[i]

Treatment[ii]	Germination (%)	Thousand seed wt (g)	MLIT (d)[iii]	Abnormal sprouts (%)	Decayed seeds (%)	Nongerminated seeds (%)
			2019			
NI	82.44 ± 1.72	15.80 ± 0.46	9.05 ± 0.14	7.15 ± 0.82	7.28 ± 1.20 a	3.14 ± 0.41
FC	84.50 ± 2.90	15.93 ± 0.23	9.19 ± 0.17	9.50 ± 1.88	3.00 ± 0.53 b	3.00 ± 0.93
IS	85.74 ± 1.86	14.62 ± 0.80	8.93 ± 0.30	5.36 ± 1.11	7.15 ± 1.33 ab	1.75 ± 0.80
SM110	87.50 ± 1.12	15.27 ± 0.58	8.70 ± 0.32	7.50 ± 1.05	3.75 ± 0.59 ab	1.25 ± 0.53
P value	0.301	0.354	0.519	0.183	**0.022**	0.107
			2020			
NI	81.70 ± 1.19	10.51 ± 0.13	8.82 ± 0.17 b	8.70 ± 0.86	2.30 ± 0.59 ab	7.30 ± 0.59
FC	79.76 ± 1.31	10.45 ± 0.17	9.93 ± 0.21 a	8.68 ± 0.90	3.48 ± 0.61 ab	8.08 ± 0.90
IS	79.21 ± 1.44	10.71 ± 0.13	10.21 ± 0.21 a	9.19 ± 0.95	4.20 ± 0.63 a	7.40 ± 0.97
SM75	80.50 ± 1.46	10.09 ± 0.22	9.98 ± 0.12 a	8.50 ± 1.26	2.40 ± 0.69 ab	8.60 ± 1.27
SM100	83.10 ± 1.41	10.46 ± 0.20	10.10 ± 0.14 a	6.50 ± 1.02	4.30 ± 0.92 a	6.10 ± 0.93
SM125	82.21 ± 1.65	10.32 ± 0.17	9.74 ± 0.16 a	9.19 ± 1.32	1.10 ± 0.42 b	7.50 ± 0.82
SM150	82.70 ± 1.29	10.11 ± 0.13	9.53 ± 0.25 ab	8.20 ± 0.74	2.20 ± 0.71 ab	6.90 ± 0.80
P value	0.336	0.116	**<0.001**	0.569	**0.007**	0.602
			2021			
NI	60.08 ± 2.10 c	14.39 ± 0.24 a	7.54 ± 0.10 b	10.92 ± 1.29 ab	27.90 ± 2.11 a	1.10 ± 0.37
FC	63.19 ± 2.24 bc	14.32 ± 0.23 a	7.76 ± 0.10 ab	12.67 ± 1.06 a	22.46 ± 1.91 ab	1.68 ± 0.54
IS	74.00 ± 2.06 a	14.45 ± 0.22 a	8.15 ± 0.18 a	9.49 ± 1.02 ab	16.11 ± 1.69 b	0.40 ± 0.28
SM75	72.01 ± 2.14 a	13.02 ± 0.22 b	7.83 ± 0.10 ab	8.51 ± 1.01 ab	18.98 ± 1.58 b	0.50 ± 0.35
SM100	72.16 ± 1.54 a	13.50 ± 0.24 ab	7.85 ± 0.11 ab	8.10 ± 0.87 b	19.24 ± 1.15 b	0.50 ± 0.29
SM125	71.18 ± 1.55 ab	13.77 ± 0.22 ab	8.31 ± 0.18 a	9.21 ± 0.99 ab	19.00 ± 1.06 b	0.60 ± 0.29
SM150	67.19 ± 1.92 a–c	14.43 ± 0.35 a	7.77 ± 0.11 ab	10.83 ± 1.17 ab	21.48 ± 1.82 ab	0.50 ± 0.25
P value	**<0.001**	**<0.001**	**0.001**	**0.043**	**<0.001**	0.103

[i] Values shown are the mean ± standard error. When P values are less than 0.05 (indicated in bold type), values followed by the same lowercase letter are not statistically separable based on Tukey's honestly significant difference.

[ii] Irrigation treatments applied to a spinach seed crop in Mount Vernon, WA, USA. Treatments were no irrigation (NI), a farmer's control (FC) based on the practices of the nearest farmer with the same parent line and a similar planting date and soil type, irrigation based on the decision support tool Irrigation Scheduler (IS) from Washington State University's Ag Weather Net, and four soil matric potential thresholds for irrigation based on soil moisture sensors: –75 (SM75), –100 or –110 (SM100 or SM110, respectively), –125 (SM125), and –150 kPa (SM150).

[iii] Mean length of incubation time (MLIT) was calculated as the product of the number of days since the start of the germination assay at each time point and the percentage of germinated seeds at that time point, summed for each time point measured, and divided by the sum of the percentage of germinated seeds at each time point.

Table 6. Leaf nutrient values as impacted by irrigation scheduling treatments.[i]

Treatment[ii]	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	S (%)	Zn ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)	Mn ($mg \cdot kg^{-1}$)	Cu ($mg \cdot kg^{-1}$)	B ($mg \cdot kg^{-1}$)
						2020						
NI	4.48 ± 0.28	0.24 ± 0.03	7.28 ± 0.43	2.31 ± 0.15	1.11 ± 0.08	0.02 ± 0.01	0.37 ± 0.01	111 ± 12	284 ± 7 b	247 ± 39 a	12 ± 0	116 ± 24
IS	4.79 ± 0.14	0.27 ± 0.01	8.12 ± 0.19	2.10 ± 0.15	1.02 ± 0.04	0.02 ± 0.01	0.41 ± 0.02	92 ± 6	429 ± 55 a	125 ± 13 b	12 ± 0	77.0 ± 9
P value	0.351	0.438	0.117	0.344	0.318	0.833	0.137	0.203	**0.031**	**0.018**	0.724	0.163
						2021						
NI	3.73 ± 0.12 b	0.27 ± 0.01	5.62 ± 0.20	2.13 ± 0.09 b	0.65 ± 0.02 b	—	0.37 ± 0.01	263 ± 19	304 ± 31 ab	255 ± 14	19 ± 0	42 ± 4
FC	4.62 ± 0.11 a	0.33 ± 0.03	5.64 ± 0.29	2.75 ± 0.17 a	0.92 ± 0.05 a	—	0.38 ± 0.01	192 ± 28	241 ± 24 b	239 ± 10	15 ± 2	41 ± 5
IS	4.70 ± 0.09 a	0.33 ± 0.02	5.54 ± 0.22	2.75 ± 0.18 a	0.89 ± 0.02 a	—	0.39 ± 0.01	191 ± 17	254 ± 31 b	249 ± 25	17 ± 1	36 ± 3
SM75	4.67 ± 0.15 a	0.32 ± 0.03	5.82 ± 0.35	2.82 ± 0.07 a	0.97 ± 0.07 a	—	0.40 ± 0.02	201 ± 22	250 ± 17 b	245 ± 9	17 ± 1	45 ± 1
SM100	4.53 ± 0.03 a	0.31 ± 0.01	6.23 ± 0.43	2.72 ± 0.05 a	0.89 ± 0.03 a	—	0.39 ± 0.02	231 ± 29	299 ± 21 ab	260 ± 12	17 ± 1	37 ± 5
SM125	4.58 ± 0.09 a	0.32 ± 0.01	6.40 ± 0.35	2.87 ± 0.08 a	0.92 ± 0.04 a	—	0.38 ± 0.00	220 ± 17	330 ± 19 ab	278 ± 7	17 ± 1	38 ± 4
SM150	3.75 ± 0.17 b	0.30 ± 0.02	5.94 ± 0.39	2.19 ± 0.10 b	0.68 ± 0.03 b	—	0.35 ± 0.02	273 ± 23	440 ± 78 a	279 ± 19	18 ± 1	46 ± 5
P value	**<0.001**	0.418	0.467	**<0.001**	**<0.001**	—	0.197	0.076	**0.011**	0.401	0.362	0.525

[i] Values shown are the mean ± standard error. When *P* values are less than 0.05 (indicated in bold type), values followed by the same lowercase letter are not statistically separable based on Tukey's honestly significant difference.

[ii] Irrigation treatments applied to a spinach seed crop in Mount Vernon, WA, USA. Treatments were no irrigation (NI), a farmer's control (FC) based on the practices of the nearest farmer with the same parent line and a similar planting date and soil type, irrigation based on the decision support tool Irrigation Scheduler (IS) from Washington State University's AgWeatherNet, and four soil matric potential thresholds for irrigation based on soil moisture sensors: −75 (SM75), −100 or −110 (SM100 or SM110, respectively), −125 (SM125), and −150 kPa (SM150).

and *S. vesicarium*. Values ranged from 60% of seeds (*SE* = ±6%) in the IS treatment to 77% (*SE* = ±5%) in the SM150 treatment. In contrast, irrigation treatments did affect the presence of morphological features identifiable as *Verticillium* spp. (P = 0.017), with increased irrigation resulting in greater incidences of *Vericillium* spp. The treatment with the lowest incidence was FC at 15% ± 9%; the treatment with the greatest incidence was SM100 at 50% ± 12% (Table 8).

Discussion

Moderating climatic and cultural factors. Although some response variables exhibited fairly consistent trends in their response to irrigation across years (e.g., vegetative biomass), others showed much more variation between years. For example, marketable seed yield was unaffected by irrigation treatments in 2019, increased consistently with each increase in irrigation frequency and amount in 2020, and increased with irrigation in 2021, but only relative to no irrigation or the treatment in which irrigation was delayed the longest (SM150) (Fig. 2; Table 3). This differential response can likely be attributed to differences in weather, parent lines, planting dates, and planting densities between years.

Weather patterns impacted the timing, and therefore also the quantity, of irrigation each year and, by extension, the effects of the irrigation treatments on the spinach crop. For example, the precipitation received in 2019 after 1 Apr was within 1 standard deviation (*SD*) of the 28-year average, and the longest stretch without precipitation was 15 d (Fig. 1), which likely led to the lack of a response to irrigation (Table 2). In contrast, although the precipitation received in 2020 was above average, especially during the early part of vegetative growth in June, the longest stretch without precipitation was 25 d in mid-July. This began right at the start of bolting, which may have been a crop growth phase that was more sensitive to moisture stress. Water restriction beginning at bolting has been shown to reduce the biomass and seed production of lettuce plants grown in a greenhouse, whereas water restriction after flowering has less consistent effects on lettuce seed weight (Contreras et al. 2008). Similarly, moisture stress applied between bolting and flowering reduced lettuce seed yield relative to moisture stress during vegetative growth (Izzeldin et al. 1980). There was an even longer stretch without precipitation in 2021 (49 d starting in mid-June, just 4 d before bolting), and the precipitation received after 1 Apr was more than 1 *SD* below the 28-year average. As such, it is unsurprising that a response of marketable seed yield to irrigation was observed in 2020 and 2021 but not in 2019. These weather patterns also explain the differences in the total irrigation applied to treatment groups and the number of irrigation events between 2019 vs. 2020 and 2021 (Table 1).

The differences among years in the marketable seed yield response to irrigation is also likely explained in part by differences in parent lines, planting dates, and planting

Table 7. P values for linear mixed-effects modeling of irrigation and inoculation main effects and their interaction on fungal disease incidence and severity.

Effect	Stemphylium leaf spot foliar severity 2020	Stemphylium leaf spot foliar severity 2021	Downy mildew incidence	*Stemphylium* spp. seed incidence	*Verticillium* spp. seed incidence[i]
Treatment[ii]	**<0.001**	**<0.001**	**0.009**	0.222	**0.008**
Inoculation	0.245	**<0.001**	—	—	—
Treatment × inoculation	0.988	**0.003**	—	—	—

[i] Includes replicates 2 through 5 only.

[ii] Irrigation treatments applied to a spinach seed crop in Mount Vernon, WA, USA. Treatments were no irrigation, a farmer's control based on the practices of the nearest farmer with the same parent line and a similar planting date and soil type, irrigation based on the decision support tool Irrigation Scheduler from Washington State University's AgWeatherNet, and four soil matric potential thresholds for irrigation based on soil moisture sensors: –75, –100 or –110, –125, and –150 kPa.

densities. The female parent line used in 2019 was a longer-standing variety (bolted 59 d after planting) compared with the quicker bolting varieties planted in 2020 and 2021 (bolted 41 and 40 d after planting, respectively). The conventional wisdom among spinach seed industry representatives is that early irrigation and adequate soil moisture is more important for quick-bolting varieties, which need to build a sufficient scaffold before setting seed, whereas early irrigation in long-standing varieties can lead to excessive vegetative growth and delayed maturity. Although the combination of the weather patterns and the parent line in 2019 likely explains the lack of a response to irrigation, these factors do not explain why a more pronounced response of marketable seed yield to irrigation was observed in 2020 vs. 2021 despite drier conditions in 2021 and similar days to bolting of the parent lines in each year. This latter difference likely can be attributed to the abnormally late planting date and greater planting density in 2020 (resulting from poor initial establishment and subsequent replanting). Therefore, despite the wet June in 2020, the greater density planting likely mined soil moisture more quickly, and the later planting date meant that plants were in an earlier growth stage when drier weather patterns arrived (Figs. 1 and 2).

Relative sensitivity of vegetative and reproductive growth to irrigation. Vegetative growth, as measured by percent canopy cover and vegetative biomass, generally followed the trend we hypothesized, with earlier and more frequent irrigation producing larger plants (Table 3). However, as discussed earlier, the response of marketable seed yield to irrigation was less consistent, and in all 3 years of the study, it was less responsive to irrigation than vegetative growth. The impact of earlier and more frequent irrigation on the relative allocation of resources to vegetative vs. reproductive growth is clearly shown by the harvest index, which decreases with increasing irrigation in 2 of the 3 years, albeit with a few exceptions (Table 3). This lower sensitivity of marketable seed yield to irrigation suggests that 1) increasing plant size and canopy cover is not an acceptable proxy for increasing seed yield, and 2) that research on irrigation in vegetable crops is unlikely to translate to the respective vegetable seed crops.

Studies on leaf spinach under greenhouse (Ekinci et al. 2015; Nasarullah et al. 2022; Nishihara et al. 2001; Seymen 2021) and field conditions (Leskovar and Piccinni 2005; Zhang et al. 2014) all show that no or very mild moisture deficits during early vegetative growth produced the highest biomass, consistent with the responsiveness of vegetative growth to irrigation in our study. However, contrary to the lower responsiveness of marketable seed yield in our study, studies in lettuce seed (Contreras et al. 2008; Izzeldin et al. 1980) and carrot seed (Steiner et al. 1990) found that seed yield was similarly sensitive to moisture stress. For example, Izzeldin et al. (1980) observed that moisture stress resulted in similar or greater decreases in lettuce seed yield than in plant biomass. Similarly, Contreras et al. (2008) saw that decreases in plant biomass and seed yield per plant resulting from moisture stress were comparable, resulting in a nearly identical harvest index. Importantly, both of these latter studies were conducted in a greenhouse, and the degree of moisture stress imposed was quite severe, such as treatments in which

Table 8. Disease incidence and severity as impacted by irrigation scheduling treatments.[i]

Treatment[ii]	Stemphylium leaf spot foliar severity[iii] Inoculated	Stemphylium leaf spot foliar severity[iii] Uninoculated	Downy mildew incidence (% leaves infected)	Seed transmission (% seed infected) *Stemphylium* spp.	Seed transmission (% seed infected) *Verticillium* spp.[iv]
2020					
NI	2.7 ± 0.3 ab	2.5 ± 0.3 a	—	—	—
FC	2.9 ± 0.6 a	2.6 ± 0.3 a	—	—	—
IS	1.3 ± 0.2 b	1.0 ± 0.0 c	—	—	—
SM75	1.7 ± 0.4 ab	1.6 ± 0.2 bc	—	—	—
SM100	1.6 ± 0.2 ab	1.7 ± 0.2 a–c	—	—	—
SM125	2.7 ± 0.3 ab	2.3 ± 0.3 ab	—	—	—
SM150	1.9 ± 0.1 ab	2.1 ± 0.1 ab	—	—	—
P value	**0.006**	**<0.001**	—	—	—
2021					
NI	65.3 ± 2.6 a	54.7 ± 2.2 a	10.0 ± 4.7 b	64.8 ± 3.3	17.5 ± 8.7
FC	40.0 ± 2.9 b	30.7 ± 3.7 b	7.5 ± 5.0 b	63.6 ± 3.5	15.0 ± 8.7
IS	36.0 ± 4.0 b	18.7 ± 3.4 b	30.0 ± 9.4 ab	60.4 ± 6.0	34.8 ± 12.6
SM75	35.7 ± 2.9 b	16.3 ± 1.7 b	35.0 ± 6.1 ab	67.2 ± 4.8	49.5 ± 8.8
SM100	29.7 ± 2.5 b	12.7 ± 1.3 b	50.0 ± 10.5 a	73.6 ± 3.4	49.8 ± 12.2
SM125	32.7 ± 2.1 b	19.3 ± 2.2 b	42.5 ± 10.2 ab	70.4 ± 6.5	49.3 ± 7.5
SM150	42.0 ± 4.6 ab	25.3 ± 3.5 b	27.5 ± 12.8 ab	76.8 ± 4.9	17.0 ± 5.4
P value	**0.001**	**<0.001**	**0.017**	0.233	**0.017**

[i] Values shown are the mean ± standard error. When P values are less than 0.05 (indicated in bold type), values followed by the same lowercase letter are not statistically separable based on Tukey's honestly significant difference.

[ii] Irrigation treatments applied to a spinach seed crop in Mount Vernon, WA, USA. Treatments were no irrigation (NI), a farmer's control (FC) based on the practices of the nearest farmer with the same parent line and a similar planting date and soil type, irrigation based on the decision support tool Irrigation Scheduler (IS) from Washington State University's AgWeatherNet, and four soil matric potential thresholds for irrigation based on soil moisture sensors: –75 (SM75), –100 (SM100), –125 (SM125), and –150 kPa (SM150).

[iii] A 1- to 5-point scale was used in 2020. Percentage of leaf coverage was used in 2021.

[iv] Includes replicates 2 through 5 only. Although the analysis of variance detected an effect of irrigation treatments, differences between individual treatments could not be identified because of the conservative nature of the Tukey means separation that was used to control the maximum experiment-wise error rate.

the soil matric potential was maintained at –500 kPa (Izzeldin et al. 1980). Interestingly, under field conditions, Steiner et al. (1990) found that the harvest index generally declined with increasing irrigation, indicating a greater response of vegetative growth than of marketable seed yield to irrigation, as observed in our study.

The lower sensitivity of marketable seed yield to irrigation and soil moisture in our study also translated to lower sensitivity of seed quality, particularly germination and seed weight. Only in 2021, the driest year, was there any effect of irrigation quantity and frequency on either of these metrics, and the percentage increases from the lowest to highest values were small in comparison with vegetative growth or even marketable seed yield (23% and 11% for germination and thousand seed weight, respectively; Table 4). Although the speed of germination (MLIT) differed among irrigation treatments in both 2020 and 2021, the changes were also relatively small (1.4 and 0.6 d, respectively). Interestingly, percent germination and thousand seed weight displayed opposite trends, with percent germination increasing with irrigation and seed weight decreasing. Similarly, Izzeldin et al. (1980) and Contreras et al. (2008) found that lettuce seed weight decreased with increasing irrigation, although the effects of moisture stress on percent germination were relatively small and less consistent. The speed of germination displayed a similar trend to thousand seed weight in 2021, with both decreasing with increasing irrigation.

Water–nutrient interactions. In both years in which leaf tissue nutrient concentrations were measured, moisture stress resulting from later and fewer irrigation events or no irrigation altered the concentrations of key plant essential nutrients, albeit in very different ways each year (Table 6). In 2020, earlier and more frequent irrigation decreased leaf tissue Mn concentrations. Available soil Mn levels are usually limited in spinach seed production because of the relatively high pH maintained for suppression of *Fusarium oxysporum* f. sp. *spinaciae* (Gatch and du Toit 2017). Given that Mn is immobile within plants (Havlin et al. 2014), it is possible that the increased vegetative growth with more irrigation in 2020 led to a dilution effect and lower Mn concentrations in the recently matured leaves that were sampled. In contrast, despite visual observation of N deficiency symptoms on the older leaves of the less irrigated plants, no differences in N concentration were detected on the recently matured leaves sampled, likely as a result of N mobility within the plant (Havlin et al. 2014).

The increased concentrations of N, Ca, and Mg in the leaf tissue of plants that received earlier and more frequent irrigation in 2021 demonstrate the importance of soil moisture in the root zone given their uptake by mass flow (Havlin et al. 2014). The two irrigation treatments that had received the least irrigation at the time of leaf sample collection (NI and SM150) had lower N, Ca, and Mg concentrations than all other treatments in 2021. Clearly, sufficient soil moisture is necessary for uptake of these nutrients, although additional irrigation does not increase the nutrient concentrations further if soil moisture is sufficient (e.g., IS or SM75 vs. SM100, SM125, or FC). Importantly, given the adequate soil and plant tissue N concentrations in the more irrigated treatments and the limiting effect of soil moisture on nutrient uptake, short of foliar fertilizer applications, in-season fertilization is unlikely to ameliorate nutrient deficiencies without additional irrigation.

Irrigation impacts on select spinach diseases. We hypothesized that the severity of SLS would increase with increasing irrigation as a result of increased free moisture, vegetative growth, and relative humidity in the canopy. Contrary to our hypothesis, SLS severity instead decreased with increasing irrigation in 2020 or with any irrigation in 2021. This is likely attributable to a couple factors. First, despite increased canopy cover with increasing irrigation, none of the treatments resulted in a closed canopy because of the upright nature of the parent lines used in 2020 and 2021. The maximum canopy cover measured was 60% in 2020 and 65% in 2021 (Table 3), which may not have been enough canopy cover to disrupt airflow and cause differences in relative humidity in the plant canopy (Mwakutuya and Banniza 2010). Second, plants in the less irrigated treatments showed clear evidence of moisture stress in both 2020 and 2021. The causal agents of SLS, *S. beticola* and *S. vesicarium*, are opportunistic pathogens, and therefore produced more severe disease on the plants experiencing abiotic stress. However, this does not mean that these results would hold for SLS in parent lines that produce a denser canopy, or for other foliar diseases. Indeed, although SLS decreased with increasing irrigation in the 2021 trial, downy mildew, which is caused by the obligate oomycete pathogen *P. effusa*, increased in prevalence with increasing irrigation, which was consistent with foliar diseases caused by oomycetes that are favored by wet conditions in the canopy (Choudhury et al. 2016).

This disease-specific pattern of responses to irrigation occurred in seed health assays just as it did in foliar disease ratings. *Stemphylium* spp. seedborne incidence was not correlated with irrigation applied, whereas the seedborne incidence of *Verticillium* spp. increased with increasing irrigation (Table 8). This is important context considering that seed is a major transmission pathway for both *Stemphylium* spp. and *Verticillium* spp., as well as for other fungal pathogens such as *Cladosporium variabile*, and considering that although it is actively managed by spinach seed producers with chlorine and hot water treatments, these treatments have variable efficacy (du Toit and Hernandez-Perez 2005; du Toit et al. 2010). This suggests that our hypothesis regarding the relationship between spinach fungal diseases and irrigation holds for some pathogens, and that the disease trade-offs involved with increasing irrigation need to be considered carefully with respect to the dominant pathogens of economic importance in each region.

Conclusion

Our research sought to assess different irrigation scheduling strategies for spinach seed production—specifically, a publicly available online tool for farmers (IS) and the use of soil moisture sensors to guide irrigation based on several threshold values (–75 to –150 kPa), compared with an unirrigated control and a control based on the irrigation practices of spinach seed producers in the area. Although increasing irrigation did increase vegetative growth consistent with our hypotheses, contrary to our hypotheses, this did not always translate to marketable seed yield. Furthermore, SLS did not increase with irrigation as hypothesized, although this hypothesis was supported for two other diseases. Importantly, our results illustrate that to guide irrigation scheduling for spinach seed production, it is necessary to consider the trade-offs involved in increasing irrigation given the differential impacts on vegetative growth, marketable seed yield, seed quality, and specific foliar and seedborne diseases. Furthermore, our data suggest that irrigation scheduling strategies must be sufficiently adaptable to account for different parent lines, planting dates, soil characteristics, field management practices, and weather patterns, given that no treatment studied was likely to maximize net returns or water productivity in all 3 years of our study. Although the treatments that received the most irrigation (IS and SM75) did maximize marketable seed yield in 2020, the NI and SM125 treatments performed comparably to the more irrigated treatments in 2019 and 2021, respectively, and water productivity (measured in kilograms of seed per cubic meter of water) decreased with increasing irrigation in all 3 years.

It is worth noting that although the FC treatment resulted in unnecessary irrigation in 2019 and did not maximize marketable seed yield under the unusual planting date and planting density in 2020, it resulted in comparable marketable seed yield to treatments that received five times as much irrigation in 2021 (Tables 1 and 3). One possible explanation for this is the timing of irrigation in the FC treatment. The first irrigation event was at a similar time to the SM75 treatment and much earlier than the SM150 treatment, but irrigation was discontinued much earlier in the season in the FC treatment (Fig. 2). Although isolating the effect of irrigation timing alone (keeping the quantity of irrigation constant) was beyond the scope of our study, whether earlier irrigation (e.g., prebolting) is more effective than later irrigation (e.g., after the start of seed set) merits further research. More broadly, research is needed to understand the growth phases that are most sensitive to water stress in spinach seed crops and other vegetable seed crops, especially given the clear differences in irrigation requirements for vegetative growth (comparable to leaf spinach) and reproductive growth demonstrated in our study.

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