HortScience 58(12):1526–1532. 2023. https://doi.org/10.21273/HORTSCI17399-23

Breeding Lettuce for Resistance against *Sclerotinia minor*

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Keywords. breeding, disease resistance, genetics, *Lactuca sativa*, lettuce drop, *Sclerotinia sclerotiorum*, vegetable

Abstract. **Lettuce drop caused by *Sclerotinia minor* is a damaging disease of romaine lettuce (*Lactuca sativa* L.) production in California. Introgression of partial resistance from wild, primitive, or heirloom accessions into modern cultivars could improve integrated management approaches to the disease. Breeding methods for lettuce drop resistance are not well developed and hinder the development of new lettuce drop–resistant cultivars. The objective of this work was to develop a pedigree-based breeding method for introgression of lettuce drop resistance into modern romaine germplasm. Progeny from crosses between the partially resistant cultivar Eruption and the susceptible romaine cultivars Darkland and Hearts Delight were selected in a modified pedigree breeding scheme. Families were evaluated for disease incidence and selected for lettuce drop resistance in artificially infested field experiments conducted in the summer and fall. Infected plants of partially resistant lines commonly do not produce seed, and therefore selection of resistant plants from infested nurseries is not possible. Noninfested field experiments were used to select individual plants with improved horticultural characteristics for seed production, but from within resistant families only. Evaluation and selection of progeny using this breeding scheme occurred from the $F_{2:3}$ through the $F_{5:6}$ generations. In all generations, superior resistance was identified in the 'Eruption' × romaine crosses. The breeding scheme generated eight green romaine-type inbred lines with better resistance than the romaine parent and better head weight than 'Eruption'. Use of the new romaine lines as parents in backcrosses to romaine produced $F_{2:3}$ families with high levels of resistance. The pedigree method used in this research can be implemented with any source of resistance, but is constrained by the use of family selection and the inability to select individual plants for resistance directly. Breeding schemes that use single seed descent or molecular markers are alternative approaches that would enable selection for resistance on individual genotypes.**

Received for publication 14 Aug 2023. Accepted for publication 22 Sep 2023.

Published online 20 Nov 2023.

This research was funded in part by several grants from the California Leafy Greens Research Program to all three authors.

We thank Sharon Benzen, Jose Orozco, Rebecca Zhao, Jinita Sthapit Kandel, Alicia Scholes, and Suiry Cardoso for their help with field trials, and Rosa Marchebout for inoculum preparation.

The mentioning of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Lettuce drop is a persistent disease that causes economic loss in lettuce production around the world. In coastal California, the largest lettuce-producing region in the United States, the disease is caused predominantly by the soil dwelling fungus *Sclerotinia minor* (Subbarao 1998). The fungus infects plant crowns, roots, or senescing leaves. Infected plants display wilting, necrosis, collapse of the lettuce head, and eventually death. Dead plants are a source of long-lived sclerotia that remain in the soil for as long as 10 years and can infect subsequent crops. A multitude of control approaches have been investigated, but single-tactic approaches are generally insufficient. Resistant cultivars, if widely available, could be an important component of integrated management of the disease (Subbarao 1998).

Romaine lettuce, a market type recognized as plants with a rosette of upright leaves that are much longer than they are wide, are grown extensively in the United States. Resistance to lettuce drop among commercial romaine cultivars is generally poor, with the exception of a handful of cultivars reported by seed companies to be partially resistant. Introgression of resistance into the romaine gene pool would be beneficial for lettuce drop control in commercial production. Immunity to *S. minor* is not known in lettuce, but there are several reports of partial resistance (Abawi and Grogan 1979; Chupp and Sherf 1960; Elia and Piglionica 1964; Grube and Ryder 2004; Hayes et al. 2010; Madjid et al. 1983; Newton and Sequeira 1972; Sherf and MacNab 1986; Subbarao 1998; Whipps et al. 2002). In these cases, accessions express reduced disease incidence at market maturity compared with known susceptible or widely grown commercial cultivars. In environments with high disease pressure, all of the known sources of resistance will exhibit disease symptoms. Many of these accessions are primitive or heirloom cultivars and wild species, and the partial resistance they offer is often believed to be conditioned by undesirable plant morphological traits (Grube 2004; Hayes et al. 2010; Newton and Sequeira 1972). Rapid bolting in particular is commonly cited as a plant trait that may condition lettuce drop resistance (Grube 2004; Hayes et al. 2010; Mamo et al. 2019, 2021; Pink et al. 2022). Among slow-bolting cultivars, the small-stature dark-red cultivar Eruption expressed a high level of partial resistance that did not appear to be associated with plant morphology or bolting (Hayes et al. 2010, 2011). Genetic analysis of a recombinant inbred line population derived from a cross between the susceptible cultivar Reine des Glaces and 'Eruption' detected four quantitative trait loci (QTLs) (Mamo et al. 2019). One of the QTLs was linked or pleiotropic with the rate of bolting, although the parents and the populations are generally considered to be slow bolting. Investigation into 'Eruption' appears to show that at least a portion of its resistance is unrelated to plant morphology or bolting, and the genes controlling this resistance could be introgressed into new genotypes with romaine plant architecture.

Lettuce (*Lactuca sativa* L.) is a diploid ($2n = 2x = 18$) autogamous species, and cultivars are highly homozygous and homogenous and do not exhibit inbreeding depression (Hayes 2015; Simko et al. 2014). Breeding methods used for lettuce are those used typically for self-pollinating crops, including pure-line selection, pedigree breeding, and backcross breeding. Pedigree-based approaches are often used for introgression efforts with wide crosses in lettuce. This is a result of the ability to select for desirable horticultural traits effectively, many of which are simply inherited, from early-generation material in field experiments. For example, the short stature of 'Eruption' was found to be conditioned by a single recessive locus (Hayes et al. 2011). Selection through the $F_{2:3}$ generation in 'Eruption' × romaine crosses was sufficient to produce lines with a basic romaine architecture. Introgressing resistance to lettuce drop presents several challenges, including correlation of resistance with undesirable morphological traits, reliance on field testing for accurate measurement of resistance, the lack of protocols for high-throughput resistance testing, dependence on disease incidence in plant populations to assess resistance, heterogeneity of disease pressure in field experiments resulting from varying levels of pathogen populations, and environmental conditions that affect disease development. Furthermore, the rate of bolting can be dependent on planting date (Rosental et al. 2021), with some genotypes bolting rapidly and exhibiting lettuce drop resistance in some planting dates but not others (Mamo et al. 2021). Because of these facts, field experiments with diverse planting dates are often needed to assess lettuce drop resistance adequately. In addition, partial resistance is insufficient to protect infected plants through seed production, and resistant plants often die before producing seed. Uninfected plants that do produce seeds are likely to be disease escapes, and are therefore unlikely to be improved genetically for resistance. Consequently, breeding for lettuce drop resistance generally requires a disease-free environment for seed production, even though selection for resistance based on phenotype is impossible in these environments. Breeding approaches that incorporate molecular markers associated with low disease offer clear advantages when dealing with all these challenges; however, markers for marker-assisted selection are unavailable. The objective of this work was to develop a pedigree-based breeding method to introgress resistance from 'Eruption' into romaine germplasm.

Materials and Methods

Germplasm and population development and handling. The starting germplasm used for this research included 'Eruption' and several commercial romaine cultivars. 'Darkland', 'Green Towers', and 'Hearts Delight' are romaine cultivars that are susceptible to lettuce drop. 'Brave Heart' and 'Green Forest' are commercially available romaine cultivars that exhibit some resistance to lettuce drop. Batavia-type cultivar Reine des Glaces is highly susceptible to the disease and was used as a susceptible control. All F_1 seed from biparental crosses were produced using the method of Ryder and Johnson (1974); all F_2 and later generations were produced by allowing the plants to self-pollinate naturally. Seed from each plant was kept separate, unless otherwise noted.

Noninfested field experiments. Noninfested, disease-free field experiments were conducted with plants of the F_2, $F_{2:3}$, $F_{3:4}$, $F_{4:5}$, and $F_{5:6}$ generations to identify superior plants and to select those plants for seed production. These experiments were established in April or May in Salinas, CA, USA, and were grown using practices typical for commercial lettuce production in coastal California (Ryder 1999). Plants were grown on two seed lines 35 cm apart on a 102-cm-wide raised bed. Spacing within the seed line was 30 cm between plants. Plots of families or lines were unreplicated, and plot length and plant population size varied between years and generations. Selected plants were dug up, transplanted into 10.5-L pots in the greenhouse, and allowed to produce seed of the next generation.

Eight advanced $F_{6:8}$ lines were evaluated in two noninfested field experiments near Salinas, CA, USA, using a randomized complete block design (RCBD) with three blocks. Seeds were planted in two seed lines 35 cm apart on 102-cm-wide beds. Each plot was at least 7 m in length, thinned to the final spacing of 30 cm between plants within a seed line, resulting in more than 30 plants per plot. Ten plants per plot that were representative of the plot were harvested and weighed together (in grams). An average head weight was determined by dividing the total weight by 10. These 10 heads were cut in half vertically and the percentage of heads with tipburn symptoms was recorded. Tipburn symptoms were assessed using the descriptions from Jenni and Hayes (2010). Five of the heads were used to measure core length and head height (in centimeters). Nine heads were retained from the 2016 experiment, processed into salad, packaged in modified atmosphere packaging, and assessed for shelf life on a 0- (no decay) to 10-point (completely decayed) scale using the method of Hayes and Liu (2008).

Artificially infested field experiments. Summer and fall artificially infested field experiments were conducted at a field site located at the US Department of Agriculture (USDA), Agricultural Research Station in Salinas, CA, USA, each year of the breeding project. The material tested included families or lines of the $F_{2:3}$, $F_{3:4}$, $F_{4:5}$, and $F_{5:6}$ generations. The parents used to generate the families or lines were used as controls in all experiments. Two summer and a single fall experiment were conducted with eight $F_{6:8}$ advanced lines; a subset of four advanced lines were tested in an additional fall experiment. The advanced lines were tested with their parents, the susceptible control 'Reine des Glaces', and commercial romaine cultivars. The experiments were conducted using an RCBD with three blocks. The field site was infested annually each spring with *S. minor* isolates (BM001, BM005, BM007, and BM010). Sclerotia of *S. minor* were produced and laid into the seed line using the method of Hayes et al. (2010) at a rate of ∼360 sclerotia/m in the spring immediately before planting. The fall experiment was conducted at the same site using the residual inoculum from the summer experiment, with the addition of any sclerotia produced on plants that died from lettuce drop during the summer experiment. Entries were seeded to plots that were 5.9 m long and used production methods and plant spacing that were otherwise the same as the noninfested field experiments. Before disease onset, the number of plants per plot was recorded, which was typically 20 to 40 plants. Disease incidence was recorded as the number of wilted, collapsed lettuce heads with white mycelium and that were developing sclerotia on the plant crown. Incidence was recorded every week, starting with the first appearance of the disease and continuing until market maturity. The cumulative mortality for each plot was totaled and expressed as a proportion based on the total number of plants per plot before disease onset. Summer experiments were seeded in April or May. Disease evaluations typically began in June and continued for 5 to 6 weeks. Fall experiments were seeded in July or August, with disease evaluations beginning in late August through October, accounting for six to seven weekly evaluations.

Naturally infested field experiments. Two field experiments were conducted with $F_{6:7}$ lines in commercial lettuce fields that had natural infestations of lettuce drop. Parents of the lines and modern romaine cultivars were included. Both experiments were conducted as an augmented RCBD, where $F_{6:7}$ lines were unreplicated and distributed randomly across six blocks. Check cultivars occurred once in each block. One experiment was seeded on 1 Jul 2013 in Spreckels, CA, USA, and was evaluated for lettuce drop incidence for the 5 weeks preceding harvest, which was on 2 Sep. A second experiment was seeded on 5 Aug 2014 in Salinas, CA, USA. Disease progression was slow in this experiment, and a single disease evaluation was recorded immediately before harvest on 18 Sep. For both experiments, data on head weight and core height were collected from surviving plants. Core height was collected from five plants and then averaged into a single plot value. Head weight in the Salinas experiment was determined by weighing collectively up to 20 plants and then dividing the weight by the number of heads weighed. In the Spreckels experiment, the number of surviving heads from the entire plot was often less than 20. Therefore, for this experiment, all the surviving heads were weighed (in kilograms) and expressed as plot yield. Plot yield was not collected in the 2014 Salinas, CA, USA, experiment.

Data were analyzed using restricted maximum likelihood in Proc Mixed of SAS (ver. 9.2; SAS Institute, Cary, NC, USA) following the guidelines of Littell et al. (2006). The cumulative lettuce drop incidence (proportion mortality) was transformed to the arcsine square root scale before analysis and is reported in tables as a disease rating (DR). In the 2013 and 2014 experiments in commercial fields, the mean trait values for all breeding lines were calculated, as was the percentage of individual breeding line means with superior performance over check cultivars. In experiments with advanced breeding lines, one-sided tests were used to determine whether breeding lines means were significantly better than check cultivar means.

Results and Discussion

Selection of resistant inbred lines derived from crosses with 'Eruption'. 'Eruption' was crossed to 'Hearts Delight' and 'Darkland'.

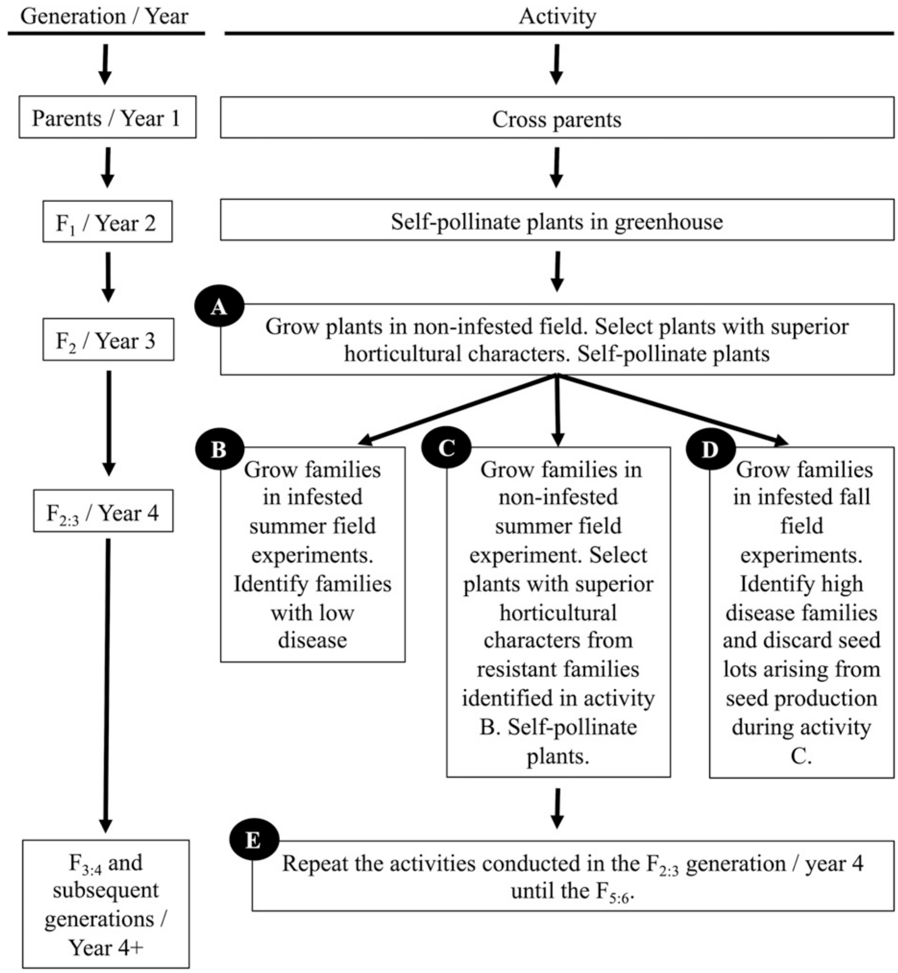

Fig. 1. Modified pedigree breeding approach to select lettuce drop–resistant inbred lines in lettuce.

Families and plants from the F_2 through $F_{5:6}$ generations originating from these crosses were evaluated for lettuce drop resistance, and horticultural characteristics in infested and noninfested field experiments using a modified pedigree-based breeding scheme (Fig. 1). After crossing the parents and self-pollinating F_1 plants, ∼500 F_2 plants were field-grown under noninfested conditions, and plants were selected that possessed horticultural characteristics that were similar to their romaine parent (Fig. 1A). The populations segregated for numerous horticultural characteristics, including but not limited to stature or size, red pigmentation, leaf pliability, leaf margin serration, leaf undulation, leaf glossiness, and darkness of green color. Plants that combined all the horticultural characteristics of the romaine parent were rare to nonexistent. Priority was given to large green plants with upright elongated leaves that were heavier, thicker, and less pliable. Given the known relationship between bolting and lettuce drop incidence, there was stringent selection against any plants that had begun to bolt at the time of selection. The selected F_2 plants were allowed to self-pollinate in a greenhouse. Starting with the $F_{2:3}$ generation and repeating in each subsequent generation, disease resistance evaluations were conducted in a summer field experiment in an infested field site to select families with a high level of resistance (Fig. 1B). In a congruent field experiment in a noninfested field site planted at about the same time, plants with superior horticultural characteristics were selected for seed production from only the families deemed resistant (Fig. 1C). The selection for horticultural traits after the $F_{2:3}$ generation used populations of ∼100 plants per family or line, and the selection criteria were similar to the F_2 generation. A fall field experiment was conducted under infested conditions to reevaluate germplasm for resistance (Fig. 1D). In some cases, families with poor resistance or poor horticultural characteristics in the summer experiments were not retested in the fall. In a limited number of cases, families were not tested in the fall because of limited seed quantities. No plants were selected and retained from the fall field experiments. Rather, we discarded the seed lots arising from plants selected from the noninfested summer field experiment that were later deemed to be susceptible. Evaluation of and selection within each generation was completed in a single year and continued with each generation until the $F_{5:6}$ generation (Fig. 1E). The final percentage

Table 1. Populations and generations of lettuce germplasm evaluated for lettuce drop resistance, number of families evaluated, number and percentage of selected families, and number of single plants from selected families in *Sclerotinia minor*–infested field experiments in Salinas, CA, USA.

	Families evaluated (n)		Selections		
			Selected families		
Population and generation	Summer	Fall	n	%	Single plants from selected families (n)
Darkland × Eruption, $F_{2:3}$	34	20	6	17.6	66
Darkland × Eruption, $F_{3:4}$	66	66	6	9.1	38
Darkland × Eruption, $F_{4:5}$	38	2	2	5.3	5
Darkland × Eruption, $F_{5:6}$	5	5	0	0	—
Hearts Delight × Eruption, $F_{2:3}$	40	40	5	12.5	78
Hearts Delight × Eruption, $F_{3:4}$	78	77	12	15.4	116
Hearts Delight × Eruption, $F_{4:5}$	116	11	6	5.2	38
Hearts Delight × Eruption, $F_{5:6}$	34	36	8	22.2	48

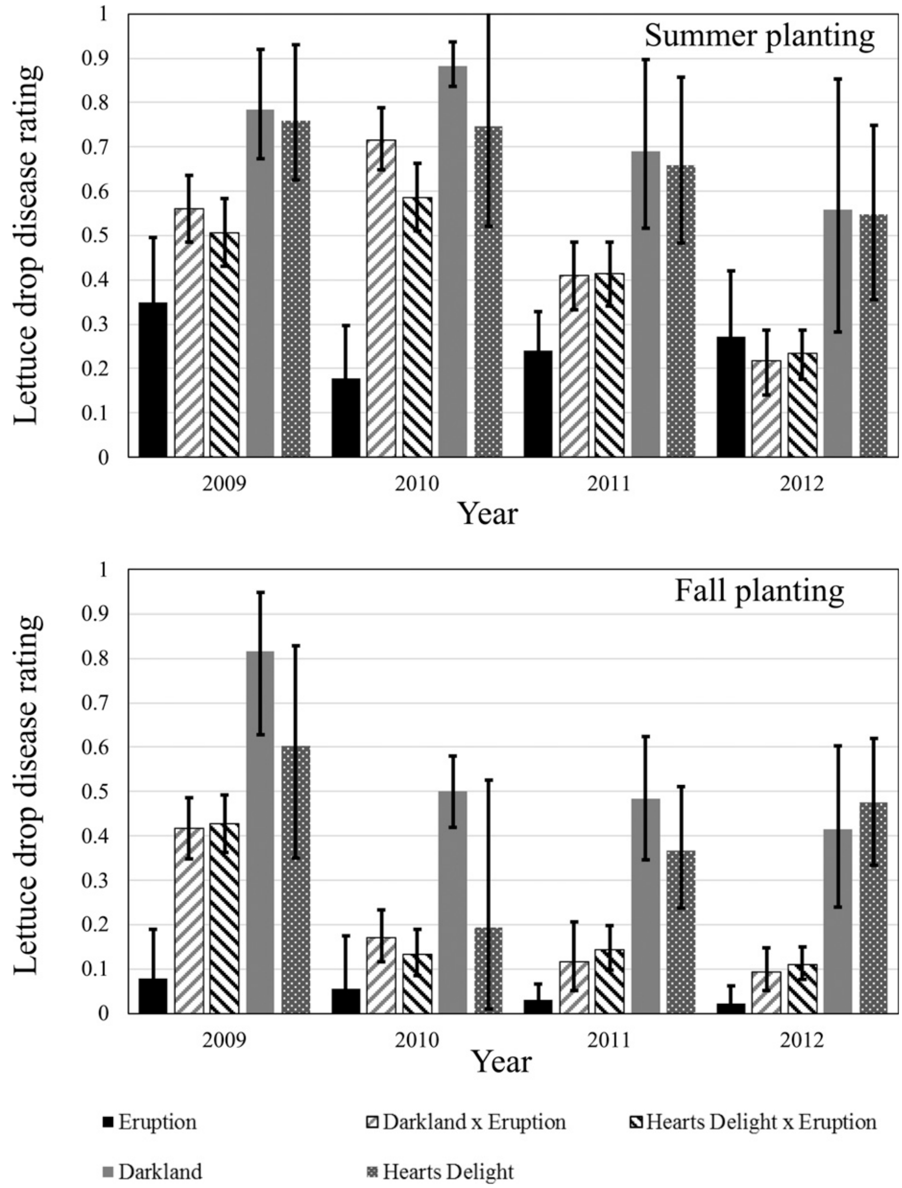

Fig. 2. Lettuce drop disease rating in summer and fall field experiments conducted in Salinas, CA, USA, in 2009, 2010, 2011, and 2012. Check cultivars were the romaine cultivars Darkland and Hearts Delight, and the small-stature dark-red cultivar Eruption. Progeny tested from 'Darkland' × 'Eruption' and 'Hearts Delight' × 'Eruption' were $F_{2:3}$, $F_{3:4}$, $F_{4:5}$, and $F_{5:6}$ in 2009, 2010, 2011, and 2012, respectively. The number of progeny families or lines evaluated in each experiment ranged from 2 to 116; the exact numbers are reported on Table 1. Disease ratings are the back-transformed values from analysis of the proportion of diseased plants on the arcsine square root scale. Experiments were conducted as a randomized complete block design with three blocks. Field site was infested with 360 sclerotia/m of *Sclerotinia minor* before planting the summer experiment. The fall experiment was conducted at the same site using the residual inoculum from the summer experiment, with the addition of any sclerotia produced on plants that died from lettuce drop during the summer experiment.

of families selected for resistance after both field experiments ranged from 0% to 22.2%, depending on the generation and pedigree (Table 1). The number of plants selected for improved horticultural characteristics ranged from 5 to 116. In general, larger percentages of families were selected from 'Hearts Delight × 'Eruption' than 'Darkland' × 'Eruption', mostly because of superior combinations of horticultural traits (data not shown). By the $F_{5:6}$ generation, the effort had developed eight $F_{5:6}$ lines from 'Hearts Delight' × 'Eruption' selected for resistance to lettuce drop. Selection and self-pollination of plants from the $F_{5:6}$ lines in a noninfested field experiment resulted in 48 $F_{6:7}$ lines. All of these lines are derived from a single $F_{3:4}$ family, numbered RH09-0488. No resistant inbred lines were derived from 'Darkland' × 'Eruption'.

The amount of lettuce drop observed in parental cultivars and the $F_{2:3}$ through $F_{5:6}$ families varied across generations and years (Fig. 2). In these experiments, disease levels were generally less in the fall than they were in the summer. This is likely explained by the practice of infesting fields artificially in the spring, and then relying on sclerotia produced from the summer experiment to cause disease in the fall experiment. The $F_{2:3}$, $F_{3:4}$, and $F_{5:6}$ generations had sufficient numbers of families or lines evaluated in summer and fall to calculate a correlation with the DR from each season. The correlation coefficients were moderate (r = 0.31, 0.51, and 0.44, respectively) but significant ($P < 0.01$), which is consistent with the variation in DR having a genetic basis (Mamo et al. 2019). 'Eruption' had a significantly lower DR than 'Hearts Delight' and 'Darkland' in all experiments, except in Summer 2012 and Fall 2010, when some comparisons were not significantly different. This further substantiates the high levels of resistance reported previously for 'Eruption' (Hayes et al. 2010, 2011; Mamo et al. 2019, 2021). Families from 'Darkland' × 'Eruption' and 'Hearts Delight' × 'Eruption' had DRs that were generally intermediate between 'Eruption' and their susceptible parent, which demonstrates that the use of 'Eruption' as a parent can result in germplasm with improved lettuce drop resistance. The populations were under continual family selection for resistance, and DRs for the two populations generally decline over years and generations. However, the project was not designed to measure gain from selection, and a decline in DR was also observed in 'Hearts Delight' and 'Darkland'. Because of these issues, it is difficult to ascertain whether the family selection method improved resistance over successive generations.

Experiments in commercial fields. The 48 $F_{6:7}$ breeding lines from 'Hearts Delight' × 'Eruption' were evaluated for lettuce drop resistance and the horticultural characteristics head weight, core height, and plot yield (2013 only) in two commercial lettuce fields with a history of lettuce drop disease. The objective of these experiments was to determine whether the experimental breeding lines were competitive with commercial romaine cultivars for critical horticultural traits and disease resistance in commercial fields, therefore making them worthy of more advanced replicated testing. Breeding lines were tested together with the cultivars Eruption, Hearts Delight, Brave Heart, Green Forest, and Green Towers. The breeding lines collectively had a mean DR of 0.21 and 0.10 in 2013 and 2014, respectively, with DRs of individual lines ranging from 0.07 to 0.42 (Table 2). The resistance in the breeding lines was superior to the cultivars (Fig. 3), and all breeding lines had less disease than the check cultivars Hearts Delight, Brave Heart, Green Forest, and Green Towers in both experiments. The two exceptions were 'Brave Heart' in 2013, where 98% of breeding lines had lower disease, and 'Green Forest' in 2014, where 96% of the breeding lines had lower disease. Head weights of the breeding lines were generally less than the check cultivars; only 2% to 6% of the breeding lines had greater head weights than the romaine check cultivars, depending on the experiment and with which check cultivars the breeding lines were compared. Conversely, all breeding lines had heads weight greater than 'Eruption'. Core heights in the breeding lines were, on average, slightly shorter than the check cultivars. Excluding 'Eruption', 75% to 100% of the breeding lines

Table 2. Lettuce drop disease rating, head weight, core height, and plot yield in 48 $F_{6:7}$ US Department of Agriculture romaine breeding lines and five cultivars in Spreckels and Salinas, CA, USA, field experiments conducted in 2013 and 2014.

		USDA breeding population[i]		Eruption		Hearts Delight		Brave Heart		Green Forest		Green Towers	
Trait	Yr	Mean	Range	Mean	Improved (%)[ii]	Mean	Improved (%)	Mean	Improved (%)	Mean	Improved (%)	Mean	Improved (%)
Disease rating[iii]	2013	0.21	0.07–0.42	0.03	0	0.69	100	0.41	98	0.47	100	0.89	100
	2014	0.10	0.07–0.19	—[iv]	—	0.21	100	0.19	100	0.17	96	0.26	100
Head weight (kg)	2013	0.90	0.7–1.2	0.4	100	1.2	2	1.1	6	1.2	2	1.2	2
	2014	0.70	0.5–0.9	—	—	0.9	2	0.9	2	0.9	2	0.9	2
Core height (cm)	2013	7.80	6.2–10.2	7.3	33	8.3	75	10.7	100	9.7	98	8.8	90
	2014	7.00	5.3–9	—	—	7.9	88	8.7	98	8.6	98	7.6	81
Plot yield (kg)[v]	2013	20.30	11.6–23.4	12.2	98	8.6	100	19.6	58	17.1	77	5.8	100

[i] US Department of Agriculture (USDA) breeding populations are 48 romaine breeding lines from 'Hearts Delight' × 'Eruption' selected previously for horticultural characteristics and lettuce drop resistance.

[ii] "Improved" means lines with equal or lower disease rating, greater head weight, shorted core height, and greater plot yield than the check cultivar.

[iii] Disease ratings are the back-transformed values from analysis of the proportion of diseased plants on the arcsine scale.

[iv] Em dash (—) indicates no data were collected.

[v] Weight of all healthy and commercial-criteria harvestable heads from 4.6 m of plot.

had a shorter core height than the check cultivars. The breeding lines had a mean plot yield of 20.3 kg in 2013, and individual breeding lines ranged from 11.6 to 23.4 kg. Depending on the check cultivar used in the comparison, 58% to 100% of the breeding lines had improved plot yield. These experiments demonstrate that the developed breeding lines are improved for resistance, have acceptably short cores, and the combination of resistance from 'Eruption' and head weight from 'Hearts Delight' results in improved plot yield in a diseased field.

Advanced line testing. Eight $F_{6:8}$ breeding lines were created by producing and massing seed from ∼30 randomly selected plants from eight of the $F_{6:7}$ lines for advanced testing in replicated experiments to determine the suitability of the lines for release. The lines were numbered RH16-0001, RH16-0002, RH16-0003, RH16-0004, RH16-0005, RH16-0006, RH16-0007, and RH16-0008. In three infested replicated field experiments, the breeding lines had DRs ranging from 0.28 to 0.57, and seven breeding lines had less disease than all tested romaine cultivars and 'Reine des Glaces' (Table 3). More specifically, the breeding lines had 28% to 68% less disease than 'Hearts Delight'. RH16-0004 (DR = 0.43) and RH16-0007 (DR = 0.43) had significantly less disease than 'Hearts Delight', disease in RH16-0008 (DR = 0.42) was significantly less than 'Hearts Delight' and 'Green Forest', and RH16-0001 (DR = 0.28) and RH16-0002 (DR = 0.31) had significantly less disease than all romaine cultivars. The breeding line RH16-0006 had a DR of 0.57, which was greater than 'Brave Heart'. In a fourth field experiment with only four breeding lines, lower DR values were observed in all lines. The DRs in RH16-0007 (0.15) and RH16-0001 (0.09) were significantly less than 'Hearts Delight', RH16-0003 (DR = 0.05) was significantly less than 'Hearts Delight' and 'Green Forest', and RH16-0002 (DR = 0.05) was significantly less than 'Hearts Delight', 'Green Forest', and 'Brave Heart'.

In two replicated noninfested field experiments to compare horticultural characteristics of the breeding lines to 'Hearts Delight', both head weight and head height was similar to or less than 'Hearts Delight' (Table 4). Core length was similar to or slightly longer than in 'Hearts Delight'. RH16-0006 consistently had the lowest head weight—25% and 32% lower than 'Hearts Delight'. The breeding lines were as susceptible to tipburn as 'Hearts Delight' and had similar quality of shelf life when processed into salad.

Fig. 3. Romaine lettuce cultivars Hearts Delight and Green Towers, and $F_{6:7}$ 'Eruption' × 'Hearts Delight' breeding lines RH12-3057 and RH12-3042 grown in a naturally infested lettuce drop commercial field experiment in Spreckels, CA, USA, in 2013. The experiment was seeded on 1 Jul and harvested on 2 Sep.

Use of improved breeding lines as a parent. Experiments were conducted to determine the usefulness of the experimental breeding lines as parents to develop lettuce drop–resistant romaine germplasm. Families were generated from the crosses RH09-0488 × 'Green Towers', RH09-0488 × PI 226641, and RH09-0488 × RH09–519. PI 226641 is a primitive romaine accession with solid midribs that had demonstrated resistance to lettuce drop previously (Hayes et al. 2010). RH09-519 is an $F_{3:4}$ family from 'Darkland' × 'Eruption' generated from the breeding scheme shown in Fig. 1. F_2 families from each cross were grown in a noninfested field experiment, and plants were selected for slow bolting, romaine-type architecture, and solid midribs when the trait segregated in the family. Selected plants were allowed to self-pollinate, and the resulting $F_{2:3}$ families were evaluated for lettuce drop resistance in replicated infested field experiments in 2014 and 2015 that included 'Green Towers', 'Eruption', PI 226641, and the susceptible control 'Reine des Glaces'. The results from the two field experiments correlated strongly ($r = 0.78$, $P < 0.001$). A scatterplot from the two experiments shows that the $F_{2:3}$ families all had less disease than 'Green Towers' and 'Reine des Glaces' in both field experiments, but generally more disease than 'Eruption' and PI 226641 (Fig. 4). The highest levels of resistance were generally observed in RH09-0488 × RH09-519 germplasm, although the family with the least disease was from RH09-0488 × 'Green Towers'. The results show that the best resistance was generally identified in families with two resistant parents derived from 'Eruption'. Despite this, four families from the resistant × resistant cross of RH09-0488 × PI 226641 were tested and demonstrated high amounts of disease. Families with better resistance from crosses with PI 226641 may be identified by testing a larger

Table 3. Lettuce drop disease rating for five cultivars and eight inbred ($F_{6:8}$) breeding lines from 'Hearts Delight' × 'Eruption' in four Salinas, CA, USA, field experiments over 3 years.

Cultivar or breeding line	Expts. 1, 2, and 3[i] Lettuce drop disease rating[ii]	Expts. 1, 2, and 3[i] Reduction in disease rating compared with Hearts Delight (%)	Expt. 4 Lettuce drop disease rating	Expt. 4 Reduction in disease rating compared with Hearts Delight (%)
Reine des Glaces	0.75		0.37	
Brave Heart	0.53		0.18	
Eruption	0.14		0.03	
Green Forest	0.60		0.23	
Hearts Delight	0.65		0.37	
RH16-0001	$0.28^{\mathrm{BH,GF,HD}}$	57	0.09^{HD}	76
RH16-0002	$0.31^{\mathrm{BH,GF,HD}}$	68	$0.05^{\mathrm{BH,GF,HD}}$	86
RH16-0003	0.49	40	$0.05^{\mathrm{GF,HD}}$	86
RH16-0004	0.43^{HD}	49		
RH16-0005	0.48	42		
RH16-0006	0.57	28		
RH16-0007	0.43^{HD}	49	0.15^{HD}	59
RH16-0008	$0.42^{\mathrm{GF,HD}}$	51		

[i] Mean shown for Expts. 1, 2, and 3 from a combined analysis; means from Expt. 4 are from a separate analysis.

[ii] Mean comparisons were done using a one-sided test between breeding lines and romaine cultivars. HD = 'Hearts Delight'; GF = 'Green Forest'; BH = 'Brave Heart'. HD, GF, and BH indicate significantly less disease than the cultivar at $P < 0.05$. Disease ratings are the back-transformed values from analysis of the proportion of diseased plants on the arcsine scale.

number of families. Alternatively, the poor performance of these families may be related to bolting. PI 226641 is a relatively rapid bolting accession identified as a possible source of lettuce drop resistance through statistical approaches (Hayes et al. 2010). It is possible that resistance in PI 226641 is dependent mostly on genes conferring rapid bolting, and the poorer resistance in these lines resulted from prior selection for slow bolting. Regardless, this research demonstrates that, when used as a parent, breeding lines derived from 'Eruption' can generate progeny families with improved levels of resistance.

Conclusion

Several years of testing in an artificially infested field determined that cultivar Eruption has the overall highest level of resistance to lettuce drop from a panel of 492 diverse accessions of *L. sativa* and four accessions of *Lactuca serriola* (Simko et al. 2023). The current research demonstrated that selection of resistant inbred lines with romaine horticultural characteristics from crosses between a romaine cultivar and 'Eruption' is possible, further substantiating the usefulness of lettuce drop resistance in 'Eruption'. RH16-0001, RH16-0002, RH16-0003, RH16-0004, RH16-0005, RH16-0007, and RH16-0008 were released as germplasm because of their improved resistance to lettuce drop compared with currently available romaine cultivars. They are available through the USDA Germplasm Resources Information Network (PI 693946–PI 693952). RH16-0006 was not released because of its low head weight and inferior resistance. The lines were released publicly with no intellectual property protection and should be used as parents to develop romaine cultivars with improved resistance. The leaves are light green and the top of the heads remain open to semiclosed at maturity. Seed color is white. The released breeding lines are romaine-type lettuce, but they have a novel appearance that is unlikely to be suitable for commercial production. It is requested that appropriate recognition be made if these populations contribute to research or the development of new germplasm, breeding lines, or cultivars.

Improvements to lettuce drop breeding approaches are needed to exploit fully the available sources of resistance. In the effort reported here, the development of romaine-type plants using a pedigree-based approach occurred within a few generations. However, pedigree breeding is limited by the inability to produce seed regularly from plants selected from infested nurseries, and by the necessity to rely on family selection. Family selection with the population sizes used in this research may result in low selection intensity, which can reduce genetic gains (Falconer and Mackay 1996). Selection of slow-bolting plants grown in a noninfested nursery could possibly reverse any gain from family selection if a gene or linked genes conditioning a correlation between resistance and bolting are segregating. The use of molecular markers to select for beneficial loci could improve genetic gains for resistance in breeding methods that use noninfested nurseries to grow plants for seed production (Mamo et al. 2019; Pink et al. 2022). Currently, no molecular marker assay is publicly available for selecting genotypes with improved resistance to lettuce drop. However, our recent genome-wide association study identified 19 QTLs for resistance to *S. minor* (Simko et al. 2023), the most prominent ones originating from 'Eruption'. At least some of these loci can be used to develop assays for marker-assisted selection (Simko et al. 2021). As an alternative that uses infested field experiments only, attempts to root lettuce shoots from plants grown in infested sites have been explored (Grube Sideman R, personal communication), but never fully implemented. In addition, disease-free plants from infested nurseries are typically considered escapes. Disease severity measures that can be applied to infected, individual field-grown plants have only recently been explored (Mamo et al. 2021). Combining single-plant disease severity measures with shoot tip culture might enable selection of resistant plants directly from disease nurseries. The use of single seed descent that produces uniform inbred lines before

Table 4. Mean head weight, head height, core height, tipburn, and salad shelf life in 'Hearts Delight', 'Eruption', and eight inbred ($F_{6:8}$) breeding lines from 'Hearts Delight' × 'Eruption' in replicated 2016 and 2019 field experiments in Salinas, CA, USA.

Cultivar or breeding line	2016 Head wt (g)	2016 Head ht (cm)	2016 Core ht (cm)	2016 Tipburn (%)	2016 Salad shelf life (0–10)[i]	2019 Head wt (g)	2019 Head ht (cm)	2019 Core ht (cm)	2019 Tipburn (%)
Eruption	510	16.7	6.9	57	6.4	410	13.7	4.3	0
Hearts Delight	1380	31.9	9.6	100	5.2	990	30.7	9.4	36
RH16-0001	1390^{HE}[ii]	29.6^{HE}	8.8^{HE}	97^{HE}	5.3	940	29.3^{HE}	$12.5^{\mathrm{HE,HHD}}$	18
RH16-0002	1500^{HE}	32.0^{HE}	10.0^{HE}	100^{HE}	5.3	750	28.4^{HE}	10.2^{HE}	0
RH16-0003	1140^{HE}	29.7^{HE}	9.5^{HE}	93^{HE}	5.4	880	29.0^{HE}	11.5^{HE}	2
RH16-0004	$1060^{\mathrm{HE,\ LHD}}$	31.0^{HE}	9.1^{HE}	100^{HE}	6.1	900	29.1^{HE}	11.0^{HE}	4
RH16-0005	1150^{HE}	30.2^{HE}	8.7^{HE}	90^{HE}	4.7^{LE}	830	$27.1^{\mathrm{HE,LHD}}$	8.9^{HE}	0
RH16-0006	$930^{\mathrm{HE,\ LHD}}$	30.2^{HE}	9.9^{HE}	90^{HE}	5.2	740	28.0^{HE}	10.3^{HE}	0
RH16-0007	1310^{HE}	30.9^{HE}	9.9^{HE}	90^{HE}	6.0	900	29.0^{HE}	10.1^{HE}	15
RH16-0008	1130^{HE}	29.6^{HE}	8.2^{LHD}	90^{HE}	$3.7^{\mathrm{LE,LHD}}$	790	28.7^{HE}	9.0^{HE}	0

[i] Decay scored as 0 = 0% decay to 10 = 100% decay 28 d after processing.

[ii] Codes following means indicate significant differences. HE = significantly higher than 'Eruption', LE = significantly lower than 'Eruption', HHD = significantly higher than 'Hearts Delight', LHD = significantly lower than 'Hearts Delight'.

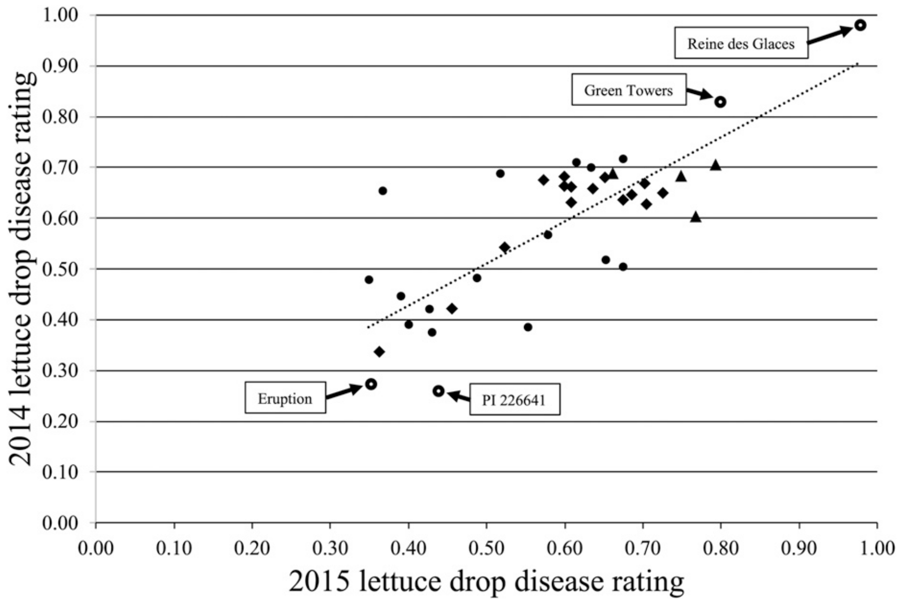

Fig. 4. Lettuce drop disease rating in 34 $F_{2:3}$ families from three crosses involving cultivar Green Towers, PI 226641, and breeding lines RH09-0488 (‘Hearts Delight’ × ‘Eruption’) and RH09-0519 (‘Darkland’ × ‘Eruption’). Commercial cultivars used as parents and the susceptible control ‘Reine des Glaces’ were included in the field experiments. Lettuce drop was evaluated in spring-planted field experiments in 2014 and 2015 in Salinas, CA, USA, and were infested artificially with 360 sclerotia/m of *Sclerotinia minor* before planting. Disease ratings are the back-transformed values from analysis of the proportion of diseased plants on the arcsine square root scale. Open circles indicate cultivars and the PI, filled circles are families from RH09-0488 × RH09-0519, filled triangles are families from RH09-0488 × PI226641, and filled diamonds are families from RH09-0488 × ‘Green Towers’.

selection is likely to be advantageous as a result of the decoupling of selection from seed production and the increased accuracy of field testing that arises from the use of inbred lines. Single seed descent routinely relies on greenhouse production of seed in small pots to develop inbred lines, which negates most chances to select for any trait. Therefore, a disadvantage of single seed descent is the need to propagate lines with undesirable horticultural traits into later generations, before they can be field tested and discarded.

References Cited

Abawi GS, Grogan RG. 1979. Epidemiology of diseases caused by *Sclerotinia* species. Phytopathology. 69:899–904. https://doi.org/10.1094/Phyto-69-899.

Chupp C, Sherf AF. 1960. Vegetable diseases and their control. Ronald Press, New York, NY, USA.

Elia M, Piglionica V. 1964. Osservazioni preliminari sulla resistenza di cultivars di lattuga ai “marciumi del colleto” da *Sclerotinia* spp. Phytopathol Mediterr. 3:37–39.

Falconer DS, Mackay TFC. 1996. Introduction to quantitative genetics (4th ed). Longman, Harlow, UK.

Grube R. 2004. Genetic analysis of resistance to lettuce drop caused by *Sclerotinia minor.* Acta Hortic. 637:49–55. https://doi.org/10.17660/ActaHortic.2004.637.4.

Grube R, Ryder EJ. 2004. Identification of lettuce (*Lactuca sativa* L.) germplasm with genetic resistance to drop caused by *Sclerotinia minor.* J Am Soc Hortic Sci. 129:70–76. https://doi.org/10.21273/JASHS.129.1.0070.

Hayes RJ. 2015. Introduction to lettuce, p 1–9. In: Subbarao KV, Gordon T (eds). Compendium of lettuce diseases. The American Phytopathological Society, St. Paul, MN, USA.

Hayes RJ, Liu Y-B. 2008. Genetic variation for shelf-life of salad-cut lettuce in modified atmosphere environments. J Am Soc Hortic Sci. 133:228–233. https://doi.org/10.21273/JASHS.133.2.228.

Hayes RJ, Wu BM, Pryor BM, Chitrampalam P, Subbarao KV. 2010. Assessment of resistance in lettuce (*Lactuca sativa* L.) to mycelial and ascospore infection by *Sclerotinia minor* Jagger and *S. sclerotiorum* (Lib.) de Bary. HortScience. 45:333–341. https://doi.org/10.21273/HORTSCI.45.3.333.

Hayes RJ, Wu BM, Subbarao KV. 2011. A single recessive gene conferring short leaves in romaine × Latin type lettuce (*Lactuca sativa* L.) crosses, and its effect on plant morphology and resistance to lettuce drop caused by *Sclerotinia minor* Jagger. Plant Breed. 130:388–393. https://doi.org/10.1111/j.1439-0523.2010.01822.x.

Jenni S, Hayes RJ. 2010. Genetic variation, genotype × environment interaction, and selection for tipburn resistance in lettuce in multi-environments. Euphytica. 171:427–439. https://doi.org/10.1007/s10681-009-0075-5.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. 2006. SAS for mixed models. SAS Institute Inc, Cary, NC, USA.

Madjid A, Honma S, Lacy ML. 1983. A greenhouse method for screening lettuce for resistance to *Sclerotinia sclerotiorum.* Sci Hortic. 18:201–206. https://doi.org/10.1016/0304-4238(83)90022-5.

Mamo BE, Eriksen RL, Adhikari N, Hayes RJ, Mou B, Simko I. 2021. Epidemiological characterization of lettuce drop (*Sclerotinia* spp.) and biophysical features of the host identify soft stem as a susceptibility factor. PhytoFrontiers. 1:182–204. https://doi.org/10.1094/PHYTOFR-12-20-0040-R.

Mamo BE, Hayes RJ, Truco MJ, Puri KD, Michelmore RW, Subbarao KV, Simko I. 2019. The genetics of resistance to lettuce drop (*Sclerotinia* spp.) in lettuce in a recombinant inbred line population from Reine des Glaces × Eruption. Theor Appl Genet. 132:2439–2460. https://doi.org/10.1007/s00122-019-03365-6.

Newton HC, Sequeira L. 1972. Possible sources of resistance in lettuce to *Sclerotinia sclerotiorum.* Plant Dis Rep. 56:875–878.

Pink H, Talbot A, Graceson A, Graham J, Higgins G, Taylor A, Jackson AC, Truco M, Michelmore R, Yao C, Gawthrop F, Pink D, Hand P, Clarkson J, Denby K. 2022. Identification of genetic loci in lettuce mediating quantitative resistance to fungal pathogens. Theor Appl Genet. 135:2481–2500. https://doi.org/10.1007/s00122-022-04129-5.

Rosental L, Still DW, You Y, Hayes RJ, Simko I. 2021. Mapping and identification of genetic loci affecting earliness of bolting and flowering in lettuce. Theor Appl Genet. 134:3319–3337. https://doi.org/10.1007/s00122-021-03898-9.

Ryder EJ. 1999. Lettuce, endive and chicory. CAB International, Wallingford, UK.

Ryder EJ, Johnson AS. 1974. Mist depollination of lettuce flowers. HortScience. 9:584. https://doi.org/10.21273/HORTSCI.9.6.584.

Sherf AF, MacNab AA. 1986. Vegetable diseases and their control (2nd ed). Wiley, Hoboken, NJ, USA.

Simko I, Hayes RJ, Mou B, McCreight JD. 2014. Lettuce and spinach, p 53–86. In: Smith S, Diers B, Specht J, Carver B (eds). Yield gains in major U.S. field crops. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI, USA. https://doi.org/10.2135/cssaspecpub33.c4.

Simko I, Jia M, Venkatesh J, Kang B-C, Weng Y, Barcaccia G, Lanteri S, Bhattarai G, Foolad MR. 2021. Genomics and marker-assisted improvement of vegetable crops. Crit Rev Plant Sci. 40:303–365. https://doi.org/10.1080/07352689.2021.1941605.

Simko I, Kandel JS, Peng H, Zhao R, Subbarao KV. 2023. Genetic determinants of lettuce resistance to drop caused by *Sclerotinia minor* identified through genome-wide association mapping frequently co-locate with loci regulating anthocyanin content. Theor Appl Genet. 136:180. https://doi.org/10.1007/s00122-023-04421-y.

Subbarao KV. 1998. Progress towards integrated management of lettuce drop. Plant Dis. 82:1068–1078. https://doi.org/10.1094/PDIS.1998.82.10.1068.

Whipps JM, Budge SP, McClement S, Pink DA. 2002. A glasshouse cropping method for screening lettuce lines for resistance to *Sclerotinia sclerotiorum.* Eur J Plant Pathol. 108:373–378. https://doi.org/10.1023/A:1015637018474.