Project Title

Investigation of a new foliar disease of lettuce caused by *Alternaria dauci*

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Abstract

In 2014 and 2015, a previously unidentified disease was found on lettuce and celery grown in the southern part of the Salinas Valley. Symptoms consisted of small (less than 1/8 inch in diameter) leaf spots that were round to oval in shape and tan to whitish in color with a brown border. Spots always remained small and hence we have referred to these as "specks." Growers and PCAs pointed out the consistent association of the problem with nearby carrot fields. Collected symptomatic lettuce and celery leaves were consistently found to be colonized with the carrot leaf blight pathogen, *Alternaria dauci*. All isolates obtained from lettuce and celery were confirmed to be *A. dauci* via morphological and molecular analyses. Inoculation experiments showed that *A. dauci* isolates from carrot, lettuce, and celery were pathogenic on all three of these crops. A field trial showed that six currently registered lettuce fungicides can protect lettuce from this speck disease. This is the first time that the carrot *A. dauci* pathogen was found to cause a disease on lettuce and celery in California.

Introduction

In the summer of 2014, reports circulated about an unfamiliar disease symptom observed on lettuce in the Salinas Valley. Symptoms consisted of small (usually less than 1/8 inch in diameter) leaf spots that ranged in shape from round to oval to irregular. Initially, these small spots were water-soaked in appearance. Later the spots turned tan to whitish in color with a brown border and were visible from both top and bottom sides of leaves. Spots always remained small and hence we have referred to these as "specks" (see photos at the end of this report). Damage, in terms of loss of quality, could be extensive since a particular leaf could have many

hundreds of specks. Specks rarely merged into each other and the leaf as a whole did not die. However, the marketability of such leaves was significantly reduced. Field samples of all types of lettuce were submitted to our lab and found to have the speck problem: iceberg, romaine, green leaf, red leaf, and oak leaf. A similar and, as we found out, related problem also occurred in celery grown in the same areas. This project was initiated to identify the cause of the specks and to devise control measures.

Objectives

1. Confirm the cause of the new speck disease of lettuce.

2. Evaluate fungicides for preventing the development of speck on lettuce.

Procedures

1. Confirm the cause of the new speck disease of lettuce.

Field surveys: Our field observations and greenhouse experiments were made possible by the critical assistance provided to us by growers and PCAs in the Salinas Valley. These industry members first pointed out the apparent association of speck damage on lettuce with nearby carrot crops that were severely diseased with Alternaria leaf blight caused by *Alternaria dauci*. Following this feedback by industry members, we conducted field surveys to document the possible role of carrot fields in this speck disease problem. From these fields we collected diseased leaves from carrot, lettuce and celery and conducted isolations in the lab to recover the possible pathogens. Fungi resembling *A. dauci* were recovered from all samples and these isolates were stored for future experiments.

Inoculations: To document the pathogenicity and host range of the carrot isolates of *A. dauci,* we purified and grew inoculum (on plates of V8 agar) of isolates obtained from a Soledad carrot field. We then sprayed the resulting spore suspension onto potted carrot, lettuce, and celery plants; plants were kept under high humidity conditions for 24 hours by placing the plants inside plastic bags. Plants were subsequently maintained in our greenhouse. In another experiment, isolates from both lettuce and celery were inoculated onto carrot, lettuce, and celery to demonstrate the cross pathogenicity of the various isolates.

Identification: Formal identification and characterization of isolates were based on both morphological and molecular assessments. Isolates from carrot, lettuce, and celery were examined morphologically with a compound microscope.

Morphological characterization. Morphological characteristics of the colony and sporulation apparatus were determined for all isolates. To characterize isolates by colony morphology, single germinating conidia were transferred to Petri dishes containing potato dextrose agar (PDA). Dishes were incubated at 22ºC in darkness for 10 days. After incubation, cultures were examined for colony color, colony margin, colony texture, and the development of pigments or crystals in the agar medium. To characterize isolates by sporulation habit, isolates were transferred to Petri dishes containing 0.05X PDA and were incubated for 7 days in a lighted

incubator. After incubation, cultures were examined at 40-100X using a dissecting microscope and substage illumination for characteristics of the sporulation apparatus, including length of conidial chains, presence elongated secondary conidiophores, and manner by which branching (if present) of conidial chains occurred. The colony and sporulation characteristics of representative cultures of *A. alternata* and *A. dauci* were also determined and compared to those of the lettuce, carrot, and celery isolates.

Molecular characteristics. For sequence analysis, the GPD gene was amplified using PCR and primers GPD1 and GPD2. DNA sequences of PCR-amplified fragments were determined with an ABI PRISM 377 DNA Sequencer using Big Dye Terminator chemistry (Perkin-Elmer/ABI, Foster City, CA). The sequences of both strands of each fragment were determined for sequence confirmation. The sequences of the isolates and the representative isolates, in addition to those from other *Alternaria* spp. obtained from GenBank, were aligned with MacClade Phylogenetic Software (version 3.05; Sinauer Associates, Sunderland, MA). In some cases, manual adjustments of sequence alignments were performed using the data editor program of MacClade. Phylogenetic analyses were performed using programs contained in PAUP Phylogenetic Software (version 4.0-beta; Sinauer Associates, Inc., Sunderland, MA). Phylogenetic trees were constructed using maximum parsimony. For parsimony analysis, heuristic searches for the most parsimonious trees were conducted using random step-wise addition of 1000 replicates and branch swapping by tree bisection-reconnection. Sequence gaps were recoded and were treated as a 5^{th} character. For statistical analyses of resolved trees, 1000 non-parametric bootstrap replicates were performed under the MP criteria.

2. Evaluate fungicides for preventing the development of speck on lettuce.

To investigate the usefulness of fungicides that could be used to manage this speck problem on lettuce, we established a replicated field trial at the USDA-ARS Spence Road station. Romaine lettuce was planted on June 10 and grown following standard practices. Beginning at the rosette stage, the lettuce was treated twice (July 23 and 30) with the fungicide treatments. On August 3 we inoculated the field with an *A. dauci* isolate obtained from carrot. A third fungicide application was made on Aug. 6 and the experiment was evaluated on Aug. 13. For the evaluation we determined the number of leaves showing specks per plant, and counted the number of specks developing in 4-cm square area on affected leaves.

Results and Discussion

1. Confirm the cause of the new speck disease of lettuce.

Field surveys: Our UCCE diagnostic lab documented that the carrot fields near damaged lettuce plantings were all infected with *Alternaria dauci*. We then conducted field surveys that confirmed grower and PCA observations that virtually all of the severe lettuce and celery speck fields were adjacent or near to these carrot fields. One fungus type was consistently recovered from carrot, lettuce, and celery leaves; this fungus resembled *A. dauci*. An important clue in diagnosing this disease is the consistent presence of dark spores in the center of the specks.

Inoculations: After 3 days, carrot plants inoculated with the carrot isolate of *A. dauci* began to show typical symptoms of Alternaria leaf blight. A few days later the lettuce and celery plants developed speck symptoms that closely resembled those observed in the field on the two respective crops. The *A. dauci* fungus was subsequently re-isolated from speck symptoms.

In the host range experiment, *A. dauci* isolates from carrot, lettuce, and celery were demonstrated to be pathogenic on all of the three tested hosts. Disease was always most severe on the carrot. Lettuce had significantly more specks than the celery.

Table 1. Host range of *A. dauci* isolates obtained from carrot, lettuce, and celery

Original host	*A. dauci* isolated	Inoculations: Pathogenicity on		
		Carrot	Lettuce	Celery
Carrot	Yes	+++	++	+
Lettuce	Yes	+++	++	+
Celery	Yes	+++	++	+

+++ = leaf blight and severe spotting symptoms
++ = moderate speck symptoms
+ = mild speck symptoms

Identification: All isolates from carrot, lettuce, and celery had spores that were brown, club-shaped with 7 to 8 transverse septa and one or more longitudinal septa per segment. Spores had the characteristic single, hyaline to pale brown, long apical beak. On PDA, all isolates produced light gray colonies that were surrounded by a violet-colored diffusible pigment in the agar medium. These features are consistent with descriptions of *A. dauci* of carrot. For the molecular analysis, a 580 bp fragment was amplified from each isolate. Multiple sequence alignments revealed 100% identity among all recovered isolates. Comparisons with other published GPD sequences of *Alternaria dauci* also revealed 100% identity, confirming the identity of all recovered isolates.

2. Evaluate fungicides for preventing the development of speck on lettuce.

For plants treated with fungicides, we found that disease incidence (Table 2: the number of diseased leaves per each plant) and disease severity (Table 3: the number of specks per 4-cm square area of leaf) were both reduced significantly for all treatments compared to the untreated control. Note that Bravo Weatherstik is not registered for use on lettuce.

Table 2. Number of leaves with specks per plant

Treatment/acre	Number of leaves w/ specks per plant
Endura 11 oz	0.0
Quadris 15.5 fl oz	0.0
Bravo Weatherstik 2 pt	0.1
Switch 14 oz	0.1
Dithane F45 1.6 qt	0.5
Fontelis 20 fl oz	1.4
Rovral 2 pt	2.0
Untreated	10.9
LSD (P = 0.05)	1.37

Table 3. Number of specks in a 4-cm square area of leaf

Treatment/acre	Number of specks per 4-cm sq
Endura 11 oz	0.0
Quadris 15.5 fl oz	0.0
Bravo Weatherstik 2 pt	1.6
Switch 14 oz	2.0
Fontelis 20 fl oz	3.9
Dithane F45 1.6 qt	7.0
Rovral 2 pt	9.9
Untreated	30.1
LSD (P = 0.05)	3.95

Conclusion

This speck disease is a new development for lettuce. There are no records of the carrot pathogen, *A. dauci*, infecting lettuce and causing damage. In addition, there is no documentation of any *Alternaria* species causing any disease on lettuce in California. Worldwide there are only a few reports of an *Alternaria* species infecting lettuce. These reports are old and from places outside of the USA; in those studies the pathogens are not well characterized or are clearly unrelated to the long-beaked *Alternaria* that we are recovering from Salinas Valley lettuce.

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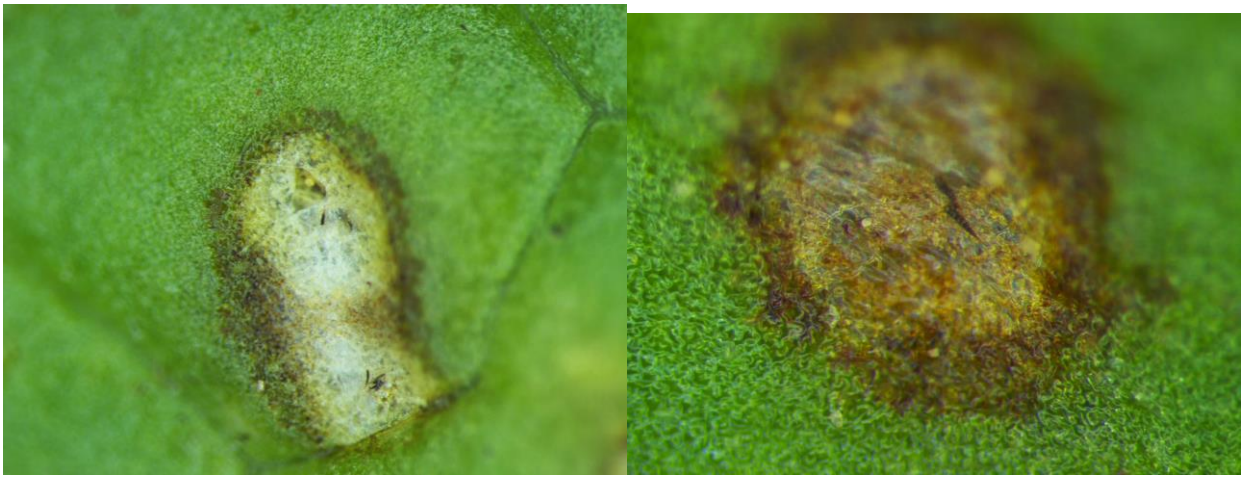
Speck symptoms on romaine lettuce.

Dissecting microscope photos showing *Alternaria* conidia in speck lesions.



Dark, elongated spore of *Alternaria dauci*.