California Pest Rating Proposal for

Lelliottia amnigena (Izard et al. 1981) Brady et al. 2013

Current Pest Rating: Q

Proposed Pest Rating: B

Kingdom: Bacteria, Phylum: Proteobacteria
Class: Gammaproteobacteria, Order: Enterobacterales
Family: Enterobacteriaceae

Comment Period: 06/07/2022 through 07/22/2022

Initiating Event:

On June 4, 2020, agricultural inspectors from Santa Barbara County submitted a sample of carrot plants (*Daucus carota*) from a field growing for export seed production. Although the above ground symptoms that triggered the sample collection were not described by the inspectors, the carrot tap roots exhibited obvious soft rot symptoms, including mucoid ooze. CDFA plant pathologist Sebastian Albu isolated the bacterium *Lelliottia amnigena* in culture. On June 26, 2020, he was able to confirm the identification of the strain with PCR, DNA sequencing, and phylogenetic analysis. On July 20, 2020, a culture was sent to the USDA's National Identifier of phytopathogenic bacteria in Riverdale, Maryland and the diagnosis was confirmed as a first detection in California. *Lelliottia amnigena* is a known pathogen of onion bulbs and strawberries, has been reported as occurring in the United States on carrot, but from an unspecified location. As this detection is the first official report in California, it was assigned a Q-rating. The risk of *L. amnigena* to California was described in a pest rating proposal that closed October 9, 2020. All pest ratings are subject to periodic review as new detection information and published literature becomes available. An updated pest rating proposal is made herein, and a new permanent rating is proposed.

History & Status:

<u>Background:</u>

Enterobacteria are the main causal agents of the economically important soft rot diseases, which lead to significant crop losses both during the growing season and during storage (Czajkowski et al., 2011). In 1981, Izard et al. described a new species in the genus *Enterobacter* that they named *E. amnigenus*.

This species is distinct from, but closely related to, *E. cloacae*, based on phenotypic and genetic studies. Their isolates were collected from drinking and surface water, and unpolluted soils. In 2013, Brady et al., based on multilocus sequence analysis, proposed that *E. amnigenus* be reclassified into *Lelliottia*, a newly described genus with three species, two of which are now known to be plant pathogens, one is not (Yuk et al., 2018). At the time of their revision, *Lelliottia amnigena* was known to be a multidrug-resistant *Enterobacter* strain capable of causing human diseases (Capdevila et al., 1998), and able to reduce nitrate to ammonia in agricultural soils (Fazzolari et al., 1990). It has recently been described as a plant pathogen by Lui et al. (2016), capable of causing decay of onion bulbs; He et al. (2017) showed it can be a damaging post-harvest pathogen of strawberry fruit, and Osai et al. (2022) completed Koch's postulates as evidence it is causing soft rot of potato tubers.

In addition to *L. amnigena*, Brady et al. (2013) also reclassified *Enterobacter nimipressuralis* as *L. nimipressuralis*. This bacterium has also been isolated from water sources and is implicated in causing wetwood disease in elm (Khodaygan et al., 2012) and wilt of *Pyracantha* (Sartori and Fucikovsky, 1976). *Enterobacter cloacae*, which is closely related to *L. amnigena*, is described in the literature as a pathogen of many crops including onion, papaya, mulberry, dragon fruit, macadamia, orchid, cassava, and chili pepper seedlings (CABI-CPC, 2020; García-González et al., 2018). It is also a seedborne pathogen of *Medicago* sp. (Zhang and Nan, 2013).

Hosts: Lelliottia amnigena has been associated with apple (*Malus domestica*), carrot (*Daucus carota* subsp. *sativus*), lettuce (*Lactuca sativa*), onion (*Allium cepa*), radish (*Raphanus sativus*), strawberry (*Fragaria × ananassa*), potato (*Solanum tuberosum*), and sugar beet (*Beta vulgaris* subsp. *vulgaris*) (Al-Kharousi et al., 2016; Cescutti et al., 2005; He et al., 2017; Hungund and Gupta, 2010; Liu et al., 2016; Osai et al., 2022). Koch's postulates have not been completed for all these hosts, but they have been for potato (Osai et al., 2022).

Symptoms: Symptoms of bacterial soft rot begin as small water-soaked lesions that can enlarge rapidly in diameter and depth. The infected interiors of plant parts become cream colored, soft, and mushy while the plant surfaces can become discolored and somewhat sunken. Tissues are slimy and disintegrate into a pulpy mass of disorganized plant cells and bacteria described as 'mucoid ooze'. The outer surface of a structure such as a carrot taproot may remain intact while the entire contents have been changed to a turbid liquid until they crack, allowing the slimy mass to exude to the surface and into the soil or storage area.

Under ideal conditions for disease, an entire fruit such as a strawberry or onion bulb may be transformed into a soft, watery, decayed mass within 3 to 5 days after initial infection. Infected fruits and tubers generally remain almost odorless until they collapse. The foul odor often accompanying soft rot infections is produced by secondary bacteria and other saprotrophic microbe invaders that further decompose plant tissue. Onions almost always give off a repulsive odor. When carrots are affected in the field, their stems may also become infected and watery, and can turn black and shrivel, causing the plants to wilt or become stunted and die (Agrios, 2005; Liu et al., 2016; He et al., 2017).

Transmission: Soft-rot bacteria may survive in infected tissues, in infected fleshy organs in storage and if left in the soil, in debris, on roots or other parts, and in contaminated equipment and containers.

They are spread by direct contact, hands, tools, soil, water, and insects. Some can overwinter in insects, in irrigation ponds and streams, and occasionally in the soil. They enter plants or plant tissues primarily through wounds or natural openings such as lenticels and hydathodes. Within the tissues they cause maceration and softening. This is accomplished by multiplying profusely in the intercellular spaces where they produce degradative enzymes, such as pectinases, proteases, lipases, and cellulases, that dissolve the middle lamella and separate the cells from one another. The plant cells first lose water and then their contents shrivel; finally, parts of their walls are dissolved, and the cells are internally invaded by the bacteria (Agrios, 2005).

Damage Potential: Bacterial soft rots cause a greater total loss of produce than any other type of bacterial disease. The formation of soft rot usually starts with the secretion of a series of exoenzymes, mainly pectinases and cellulases, which depolymerize the plant cell wall. Through the secretion of pectate lyase enzymes, polygalacturonases, pectin lyases, pectin methylesterases, cellulases and proteases, and infections by *L. amnigena* result in the maceration of parenchyma cells (Popović et al. 2017).

Maceration is the most common symptom of rotten tubers and fleshy roots. It is dependent on outdoor temperature and humidity. Different soft rotting species occur worldwide and cause serious losses in the field, in transit, and especially in storage. Nearly all fresh vegetables are susceptible to some type of bacterial soft rot, which may develop within a few hours in storage, or during transit. Although there is limited information on *L. amnigena*, related soft rotter species are known to reduce yield, quality, and the market value of crops, and greatly increase expenses for implementing preventive measures such as altered storage conditions, field irrigation, and crop rotations (Agrios, 2005).

Worldwide Distribution: China (Liu et al., 2016; He et al., 2017), Finland (Cescutti et al. 2005), India (Hungund and Gupta, 2010), Egypt, Oman, USA (Al-Kharousi et al., 2016), United States (California) PDR Database, 2020).

Official Control: This is a Q-rated pathogen in California (see 'Initiating event') and detection requires destruction of infected materials.

California Distribution: One detection has been made from carrot (2020) and one from kohlrabi (2022) in Santa Barbara County, one detection was made from Humboldt County from necrotic areas of bark tissue of *Acer* sp. (2021). *Pantoea eucalypti,* and *Pantoea agglomerans* were also detected from the diseased kohlrabi.

California Interceptions: None

The risk *Lelliottia amnigena* would pose to California is evaluated below.

Consequences of Introduction:

1) **Climate/Host Interaction:** In general, soft-rotting bacteria require wet soils or wet plant surfaces to multiply and infect. These conditions occur in greenhouses, in storage, and in irrigated agriculture statewide.

Evaluate if the pest would have suitable hosts and climate to establish in California.

Score: **2**

- Low (1) Not likely to establish in California; or likely to establish in very limited areas.
- **Medium (2) may be able to establish in a larger but limited part of California.**
- High (3) likely to establish a widespread distribution in California.

2) **Known Pest Host Range:** The host range is primarily plants with significant underground structures including carrots, onions, beets, and potatoes. It is also associated with above ground plant parts such as apples and strawberries. The host range will likely increase over time.

Evaluate the host range of the pest.

Score: 3

- Low (1) has a very limited host range.
- Medium (2) has a moderate host range.
- **High (3) has a wide host range.**

3) **Pest Reproductive Potential:** The pathogen is highly likely to spread primarily through infested soil and or water. It may have a seed pathway, either as a true seed borne pest or as a seed contaminant.

Evaluate the natural and artificial dispersal potential of the pest.

Score: 2

- Low (1) does not have high reproductive or dispersal potential.
- **Medium (2) has either high reproductive or dispersal potential.**
- High (3) has both high reproduction and dispersal potential.

4) **Economic Impact**: It is not known what effect *Lelliottia amnigena* could have on crop yield beyond potatoes. Also, it remains to be determined if management of the bacterium would change certain cultural practices to reduce/eliminate incidences and causes of spread. In general, the control of bacterial soft rotters is difficult and depends on using proper sanitation, avoiding injuries, keeping storage tissues dry and cool, practicing good insect control, and employing crop rotation.

Evaluate the economic impact of the pest to California using the criteria below.

Economic Impact: A, D, G

A. The pest could lower crop yield.
B. The pest could lower crop value (includes increasing crop production costs).
C. The pest could trigger the loss of markets (includes quarantines).
D. The pest could negatively change normal cultural practices.
E. The pest can vector, or is vectored, by another pestiferous organism.
F. The organism is injurious or poisonous to agriculturally important animals.

G. The organism can interfere with the delivery or supply of water for agricultural uses.

Economic Impact Score: **3**

- Low (1) causes 0 or 1 of these impacts.
- Medium (2) causes 2 of these impacts.
- **High (3) causes 3 or more of these impacts.**

5) **Environmental Impact: 2**

Information on the effect of *Lelliottia amnigena* on the environment is currently not known. Control of other soft rotting bacteria requires strict attention to sanitation

Environmental Impact:

A. The pest could have a significant environmental impact such as lowering biodiversity, disrupting natural communities, or changing ecosystem processes.
B. The pest could directly affect threatened or endangered species.
C. The pest could impact threatened or endangered species by disrupting critical habitats.
D. The pest could trigger additional official or private treatment programs.
E. The pest significantly impacts cultural practices, home/urban gardening or ornamental plantings.

Environmental Impact Score: 2

- Low (1) causes none of the above to occur.
- **Medium (2) causes one of the above to occur.**
- High (3) causes two or more of the above to occur.

Consequences of Introduction to California for *Lelliottia amnigena:* Medium

Add up the total score and include it here. **12**

-Low = 5-8 points

-Medium = 9-12 points

-High = 13-15 points

6) **Post Entry Distribution and Survey Information**: Evaluate the known distribution in California. Only official records identified by a taxonomic expert and supported by voucher specimens deposited in natural history collections should be considered. Pest incursions that have been eradicated, are under eradication, or have been delimited with no further detections should not be included.

Evaluation is 'low'. This disease has been found in two vegetable seed fields in Santa Barbara County on carrots and kohlrabi and one maple tree in Humboldt County.

Score: -1

-Not established (0) Pest never detected in California or known only from incursions.

-Low (-1) Pest has a localized distribution in California or is established in one suitable climate/host area (region).
-Medium (-2) Pest is widespread in California but not fully established in the endangered area, or pest established in two contiguous suitable climate/host areas.
-High (-3) Pest has fully established in the endangered area, or pest is reported in more than two contiguous or non-contiguous suitable climate/host areas.

7) **The final score is** the consequences of introduction score minus the post entry distribution and survey information score:

Final Score: *Score of Consequences of Introduction – Score of Post Entry Distribution and Survey Information* **= 11**

Uncertainty:

The host range of this pathogen could expand once more information is available on its mode of spread and if testing is done for other plant species.

Conclusion and Rating Justification:

Based on the evidence provided above the proposed rating for *Lelliottia amnigena* is B.

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Responsible Party:

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*Comment Period: 06/07/2022 through 07/22/2022

*NOTE:

You must be registered and logged in to post a comment. If you have registered and have not received the registration confirmation, please contact us at permits [@] cdfa.ca.gov.

Comment Format:

- Comments should refer to the appropriate California Pest Rating Proposal Form subsection(s) being commented on, as shown below.

  Example Comment:

  Consequences of Introduction: 1. Climate/Host Interaction: [Your comment that relates to "Climate/Host Interaction" here.]

- Posted comments will not be able to be viewed immediately.
- Comments may not be posted if they:

  Contain inappropriate language which is not germane to the pest rating proposal;

  Contains defamatory, false, inaccurate, abusive, obscene, pornographic, sexually oriented, threatening, racially offensive, discriminatory or illegal material;

Violates agency regulations prohibiting sexual harassment or other forms of discrimination;

Violates agency regulations prohibiting workplace violence, including threats.

- Comments may be edited prior to posting to ensure they are entirely germane.
- Posted comments shall be those which have been approved in content and posted to the website to be viewed, not just submitted.

Proposed Pest Rating: B