Soft Rot *Enterobacteriaceae* Are Carried by a Large Range of Insect Species in Potato Fields

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ABSTRACT Pathogenic soft rot *Enterobacteriaceae* (SRE) belonging to the genera *Pectobacterium* and *Dickeya* cause diseases in potato and numerous other crops. Seed potatoes are the most important source of infection, but how pathogen-free tubers initially become infected remains an enigma. Since the 1920s, insects have been hypothesized to contribute to SRE transmission. To validate this hypothesis and to map the insect species potentially involved in SRE dispersal, we have analyzed the occurrence of SRE in insects recovered from potato fields over a period of 2 years. Twenty-eight yellow sticky traps were set up in 10 potato fields throughout Norway to attract and trap insects. Total DNA recovered from over 2,000 randomly chosen trapped insects was tested for SRE, using a specific quantitative PCR (qPCR) TaqMan assay, and insects that tested positive were identified by DNA barcoding. Although the occurrence of SRE-carrying insects varied, they were found in all the tested fields. While *Delia* species were dominant among the insects that carried the largest amount of SRE, more than 80 other SRE-carrying insect species were identified, and they had different levels of abundance. Additionally, the occurrence of SRE in three laboratory-reared insect species was analyzed, and this suggested that SRE are natural members of some insect microbiomes, with herbivorous *Delia floralis* carrying more SRE than the cabbage moth (*Plutella xylostella*) and carnivorous green lacewing larvae (*Chrysoperla carnea*). In summary, the high proportion, variety, and ubiquity of insects that carried SRE show the need to address this source of the pathogens to reduce the initial infection of seed material.

IMPORTANCE Soft rot *Enterobacteriaceae* are among the most important pathogens of a wide range of vegetables and fruits. The bacteria cause severe rots in the field and in storage, leading to considerable harvest losses. In potato, efforts to understand how soft rot bacteria infect and spread between healthy plants have been made for over a century. Early on, fly larvae were implicated in the transmission of these bacteria. This work aimed at investigating the occurrence of soft rot bacteria in insects present in potato fields and at identifying the species of these insects to better understand the potential of this suspected source of transmission. In all tested potato fields, a large proportion of insects were found to carry soft rot bacteria. This suggests a need to give more weight to the role of insects in soft rot ecology and epidemiology to design more effective pest management strategies that integrate this factor.

KEYWORDS plant pathology, soft rot, insect vectors, potato

Soft rot *Enterobacteriaceae* (SRE) are pathogenic organisms of the genera *Pectobacterium* and *Dickeya* that cause soft rots in plant species from 50% of angiosperm plant orders, including a wide variety of economically important crops, such as potatoes, tomatoes, onions, peppers, and cabbage (1). In potatoes, SRE cause soft rot in both tubers and stems. The bacteria enter potato tubers through lenticels and through

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fresh wounds on the tubers, roots, and above-ground parts of the plant (2). Blackleg symptoms follow soft rot in an infected seed tuber piece and a subsequent spreading of the pathogen through the vascular system (3).

The SRE species responsible for the most significant pre- and postharvest losses in potato are *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *brasiliensis*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium parmentieri*, *Dickeya dianthicola*, and *Dickeya solani* (4–6). Some isolates of *Pectobacterium carotovorum* subsp. *carotovorum* were recently reclassified into the new species *Pectobacterium polaris* (7).

Infected seed tubers are considered to be the most important source of bacteria, and mechanical handling during planting and harvest contributes substantially to the spread among tubers (4, 8). The production of seed potatoes is initiated with minitubers originating from *in vitro* plant cultures that are free of SRE when planted in the field. These are multiplied in the field for economic reasons, and during multiplication, a steady increase in SRE levels can be observed in each field generation (4). The mechanisms of the initial infection of clean source material, such as tissue culture clones or stem cuttings, remain unexplained, although aerosols and insects have long been considered possible sources of SRE transmission (9, 10).

Insects function as alternative hosts and vectors of numerous phytopathogens, and various transmission systems have been identified (11). Such findings were used to develop and implement more efficient prevention strategies by targeting the insect vector instead of the pathogen, as exemplified in the control of various plant viruses through a decrease in their vectors (12).

Previous research on insects as vectors of SRE in potato has largely focused on *Delia platura* and *Drosophila melanogaster*. In the early 20th century, it was first observed that *D. platura* (seedcorn maggot) laid eggs near tubers shortly after planting. It was hypothesized that the larvae frequently transmitted SRE to the tubers by boring into them (13). Later, it was shown that artificially inoculated *D. platura* adults transmitted *P. carotovorum* subsp. *carotovorum* to healthy potato plants in a cage experiment (14). Similarly, *Delia radicum* (cabbage root fly) and *Delia antiqua* (onion fly) were shown to transmit SRE to their respective host plants (15–17). Furthermore, it was demonstrated that SRE could be transmitted from infected to healthy potato plants by *D. melanogaster* (18) and that some strains were able to survive in *Drosophila* spp. for at least 72 h (19).

In addition to the detailed work done on *Delia* and *Drosophila* species, studies of other insects potentially involved in SRE transmission were previously attempted around two potato waste dumps (9) and one field site for the propagation of clean seed material (20) in Scotland. However, since these studies were conducted in the 1970s, the detection of SRE required enrichment on artificial medium, and insect identification relied on morphological taxonomy. Despite strong indications of insects as a source of initial SRE infection in the field that was tested, the efforts were only moderately conclusive, since the isolation of bacteria was partially done from bulked insects and some that were not identified beyond their taxonomical order. The morphological identification of the few insect specimens found to carry SRE in those studies showed, among others, *Leptocera* spp., *Scaptomyza* spp., *Scatopse* spp., *Delia* spp., *Drosophila* spp., and unidentified Diptera (true fly) specimens (9, 20).

Phytopathogens that are transmitted by insect vectors can be described as having various degrees of vector specificity (21). Vector specificity is considered high if the phytopathogen is transmitted by one or a few insect species, as is the case for *Pantoea stewartii* and its vector, the corn flea beetle (11). Conversely, if a phytopathogen is transmitted by many different insect species, it has a low vector specificity; *Erwinia amylovora*, for example, is transmitted by a broad range of pollinator species (11) and has been detected in various insect pests (22). Research on insect vectors of SRE has not yielded sufficient data to address vector specificity.

The potential contribution of insects to initial SRE infection of potato and other crops, as well as to the dissemination within and between fields, remains unclear. The objective of this study, therefore, was to examine the presence of SRE in insects in

FIG 1 Map of Norway with indicators for all field locations in 2015 (O) and 2016 (X) and associated proportions of samples that tested positive (pos.; blue) and negative (neg.; gray) for SRE using the PEC assay. Names of the field locations are given above each pie chart. Overall proportions for 2015 (top), 2016 (center), and in total (bottom) are given on the right. Distances of the traps from any symptomatic plants are indicated under each pie chart. Fields with traps set up in a minimum (Min.) distance of 10 m from any symptomatic plant did not necessarily contain symptomatic plants. Further details are given in Table S1. (Map templates are from Geonorge.)

multiple potato fields by molecular methods suited for a sufficiently sensitive and efficient detection of pathogens from individual insects and more accurate identification of insect species. The test sites included fields where plants were symptomatic and one location where seed material was propagated from clean tubers generated from tissue culture. The field where clean seed tubers were grown was particularly informative in our examination of the potential of insect-borne SRE to contribute to initial infection. The chosen scope was intended to reveal new potential insect vectors that were not found in previous work and give an indication of the overall distribution of SRE over various insect species. Showing this distribution is a first step in the identification of possible vector candidates for SRE, thereby allowing for appropriate control measures to be developed.

RESULTS

A substantial proportion of insect samples contains SRE. To assess the potential of insects to present a viable inoculum for SRE transmission, individual insects sampled from potato fields in Norway using sticky traps (see Fig. S1 in the supplemental material) were examined for two consecutive years (Fig. 1).

The presence of SRE was tested by using a quantitative PCR (qPCR) assay targeting all *Dickeya* and *Pectobacterium* species (23). The threshold for a positive test (quantification cycle [C_q] < 28) was chosen conservatively to only include insects with a high load of SRE and corresponded to between 10,000 and 100,000 CFU, as determined by a dilution series experiment (Fig. S2). SRE were isolated from an insect that tested positive and caused soft rot symptoms when inoculated in SRE-free minitubers (Fig. S3).

Insects from all traps in all fields contained large amounts of SRE, with percentages ranging from 4% to 39% of insects from a given trap (Table S1). Out of 2,122 tested insects in total, 19% were positive for SRE with the chosen threshold. The overall

FIG 2 Identification, classification, and proportions of insect specimens that tested positive for SRE. (A) Genera of insect specimens that tested positive over both years (inner circle), as well as species that tested positive in 2015 (second circle), 2016 (third circle), and over both years (outer circle). Only taxa with more than 10 representatives over both years are shown, with the rest represented as “other.” (B) Insect families and orders for specimens that tested positive over both years with the most prevalent family (Anthomyiidae) and order (Diptera) are highlighted in black, and others are in light gray.

percentage of insects that tested positive varied between 15% in 2015 and 23% in 2016 (Fig. 1).

A diverse group of insect species carries SRE. The insect specimens that tested positive for SRE in the qPCR assay were regarded as potential vectors due to the large amount of bacteria they contained. Species identification of the insects by DNA barcoding was successfully performed for 367 of the 401 SRE-positive insect samples (Fig. 2A). The identified specimens belonged to at least 91 different insect species, with 95% of the identified species belonging to the order Diptera (Table S2).

The families most commonly found to carry a large amount of SRE were *Anthomyiidae*, in 46% of the identified samples, and *Muscidae*, in 14% of the identified samples (Table S2). *Delia* was the dominant genus among the samples that tested positive, with 36% in 2015, 30% in 2016, and 32% in total (Fig. 2A). The most prominent species was *D. platura*, making up 19% of all positive samples. The positive specimens collected from traps in the northernmost field (Overhalla) were dominated by *Delia coarctata*, with only one individual being identified as *D. platura*, whereas *D. coarctata* only tested positive in the other locations sporadically (Table S2). In addition to Diptera, a number of Hemiptera (true bugs), mainly leafhoppers of the species *Empoasca decipiens*, tested positive in both years.

Although the proportions of the identified species were mostly stable across years, some species varied in abundance (Fig. 2A). This likely resulted from single species, with many individuals that tested positive occurring in one of the examined locations exclusively (Table S2).

Dissemination of SRE by insects within and between fields. The SRE species *D. solani* was identified in three individual insects from one field in 2015 and isolated from a symptomatic plant in the same field that year (Table 1). The finding of *D. solani* was unexpected, since it is an invasive species that was previously detected only once in Norway, in a quarantine field with imported seed material in 2012 (24). Since then, all certified seed potato lots are tested for *D. solani*, and there have been no detections in these.

TABLE 1 SRE species detected in symptomatic plants and insects from potato fields^a

		No. of positive organisms or presence/absence of organisms detected					
Field by yr	Source	<i>Dickeya solani</i>	<i>Pectobacterium atrosepticum</i>	<i>Pectobacterium carotovorum</i> subsp. <i>brasiliensis</i>	<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	<i>Pectobacterium parmentieri</i>	PEC C_q < 28 ^b
2015							
Apelsvoll	Insects	3	6	0	NA	2	38
	Potato plants	+	+	—	—	—	
Brandval	Insects	0	6	0	NA	2	26
	Potato plants	—	+	—	—	—	
Gjervoldstad	Insects	0	24	0	NA	2	45
	Potato plants	—	+	—	—	—	
Hamar	Insects	0	11	0	NA	1	43
	Potato plants	—	+	—	+	—	
Larvik	Insects	0	0	0	NA	1	8
	Potato plants	—	+	—	—	—	
Rygge	Insects	0	1	0	NA	0	11
	Potato plants	—	+	—	+	—	
2016							
Hamar	Insects	0	0	0	NA	5	40
Overhalla	Insects	0	13	0	NA	34	103
Reddal	Insects	0	27	0	NA	10	62
Ås	Insects	0	0	0	NA	3	25

^aIdentification of SRE was done by using species-specific TaqMan assays on insects that tested positive in the PEC assay, or by FAME analysis of isolates from blackleg lesions of potato plants adjacent to traps. The numbers refer to insect specimens that tested positive for each of the species-specific TaqMan assays, for each field. The SRE species that were isolated from the symptomatic potato plants adjacent to the traps are indicated as “found” (+) and “not found” (—) based on the FAME identification. The insect data for *Pectobacterium carotovorum* subsp. *carotovorum* are not available (NA) since there was no specific TaqMan assay for it. In the 2016 fields, symptomatic potato plants were not tested.

^bThe number of insects that tested positive for SRE with the general PEC TaqMan assay in each field. Out of 401 positive insects, 10 were not tested with species-specific TaqMan assays due to a limited amount of DNA.

Insects trapped from a field dedicated to the propagation of germfree minitubers in Overhalla tested positive for SRE, with a relatively high percentage compared to the other fields where traps were tested (Fig. 1). A source of the bacteria within those fields was very unlikely due to the quality of the seed tubers.

Generally, increasing the distance of the traps from plants with blackleg did not lead to a lower percentage of insects that tested positive. Insects collected from a minimum distance of 10 m from a plant with blackleg tested positive in 22% of the samples. For insects trapped in immediate proximity of symptomatic plants, 16% samples tested positive (Table S1).

Abundance of insect species carrying SRE shows two extremes. While our data indicate a large variety of insect species to be capable of carrying large amounts of SRE, the number of individuals that tested positive differed widely between species. Two extremes were observed in the identified species, an abundance of species with few individuals that tested positive, versus few species with a large number of individuals that tested positive (Fig. 3). As the most extreme in the second group, *D. platura* alone represents one-fifth of all identified individuals. Together with nine other species, *D. platura* makes up more than 50% of the individuals shown to carry a high number of SRE. The remaining individuals belong to at least 79 different species, with eight or fewer individuals observed over both years. For 50 species, only one individual tested positive over both years (Fig. 3 and Table S2).

Laboratory-reared *Delia floralis* contains large amounts of SRE. To investigate further the relationship between SRE and *Delia* spp., we tested individuals from two generations of a long-term laboratory rearing of *D. floralis* (turnip root fly). Of these, 66% of 94 individuals tested positive for SRE using the rather conservative C_q level of 28 (Fig. 4). For comparison, we tested two other laboratory-reared insect species, *Plutella xylostella* (cabbage moth), because of its similar rearing conditions, and carnivorous *Chrysoperla carnea* (common green lacewing) larvae. The number of specimens positive for SRE was significantly higher in *D. floralis* samples than in the other tested

FIG 3 The relationship between the number of insect species and number of individuals for a given species. Number of individuals (x axis) refers to the number of instances of a species being identified, while number of identified species (y axis) refers to the number of instances where one species was identified with the corresponding amount of individuals (x axis). Samples for 2015 (O), 2016 (×), and in total (filled circles) are shown.

species, as well as in the samples trapped in the fields (Fig. 4). For *P. xylostella*, 13% of 94 of the specimens tested positive for SRE, and for the *C. carnea* larvae, only one out of 40 specimens tested positive for SRE. Furthermore, the average amount of SRE was significantly larger in *D. floralis* specimens than in *P. xylostella* and *C. carnea* specimens. Interestingly, adult individuals of both *C. carnea* and the closely related *Chrysoperla lucasina* (one each) that feed on pollen and nectar tested positive in the wild trapped samples (Table S2).

DISCUSSION

SRE have a broad host plant spectrum and can be found in rotting lesions of wild and cultivated plants (1), which might attract a variety of insects for egg deposition or feeding. It is therefore reasonable to assume that a number of different insect species encounter SRE in various amounts, depending on their behavior. The results shown in Fig. 2 support this assumption. The results suggest that Diptera are more likely to acquire or have SRE as members of their microbiome than other insects. However, the bias toward Diptera might be inherent to the sampling method in terms of the color of

FIG 4 Number of SRE in insect samples from traps and from laboratory rearings, with median (black line) and distribution of all samples. The CFU were calculated using a linear approximation for the relationship between C_q and CFU values from the dilution series data (Fig. S2); samples from $1:10^3$ to $1:10^6$ were used to create the linear approximation. For *Delia floralis*, 94 samples of adult flies from two consecutive generations were tested (47 each); for *Plutella xylostella*, 94 samples of adult moths were tested; and for *Chrysoperla carnea*, 40 samples of larvae were tested. The red line indicates the calculated CFU corresponding to the C_q 28 threshold used in the field samples. Letters a to d indicate significantly different groups of samples according to a Mann-Whitney test ($P < 0.05$); all combinations were tested for both C_q and calculated CFU values.

the sticky traps (yellow) and that they were mounted above ground, excluding ground-dwelling insect species.

More than half of the identified insect species that tested positive for SRE were represented by only one individual in both years (Fig. 3). Since only individuals that tested positive were identified, it is not possible to infer the proportion of individuals of a given species that were carrying SRE. Contamination of some individuals on the traps by aerosols or cross-contamination from other insects that carried many SRE cannot be excluded. However, aerosol contamination would in principle be expected to be higher for samples taken close to symptomatic plants, but the proportions of positive samples were comparable (Table S1). Cross-contamination was assumed to be negligible, since the sampled insects rarely were in contact with each other on the traps. Likely explanations for species testing positive in few individuals could be that these species were either not abundantly present at the time and location of the experiment, not trapped, not tested, or simply are not commonly associated with SRE. If they were not commonly associated, this would suggest that at least some of the species that tested positive and were identified might not be dedicated vectors for SRE. However, some degree of stochastic transmission from these individuals is conceivable, given a sufficient presence of insects carrying large amounts of the pathogen. SRE have been shown to cause systemic infections upon inoculation in wounded tubers, stems, and leaves of potato under suitable conditions (25). This suggests a potential mechanism for stochastic transmission of SRE by various insects that visit and cause plant wounds. In such cases, SRE could be applied to and transferred between wounds by insects that retain the bacteria on their surface or mouthparts. Alternatively, SRE could be introduced during wounding by insects that carry SRE internally for a short period.

D. platura stood out as the Diptera species that carried SRE most frequently in both years of the study. In addition, six other *Delia* species were frequent carriers of SRE, which supports earlier work done on the relationship between SRE and various *Delia* species (13, 16, 17). The ecology of *Delia* species explains the acquisition of SRE at the larval stage, either from rotten plant tissue or by vertical transmission from the mother via the egg surface (13). SRE infection of plants through *Delia* spp. has been shown from the larvae to the seed material of their host (13, 16, 17), as well as from adult flies to wounded petioles and leaves of potato plants (14). In addition to the transmission, long-term survival of SRE in the pupae of *D. platura* that overwinter buried in the soil (26) offers a favorable means for the bacteria to survive the winter in spite of prolonged freezing periods in temperate climates. Normally, SRE survive poorly in the environment in a temperate climate (27).

In addition to Diptera, some specimens of the hemipteran leafhopper *E. decipiens* tested positive for SRE. *E. decipiens* has been shown to transmit "*Candidatus* Phytoplasma asteris" to daisies by feeding on leaves (28) and has previously been described as a potato pest (29). Plant pests, like *E. decipiens*, are likely vector candidates, since leafhoppers actively damage the plant tissue by their stylet-like mouthparts that they use for sucking plant sap (30), thereby creating suitable conditions for SRE infection (25). Dedicated efforts to show the transmission of SRE to potato or other plants by the different insect species that were identified here are needed to show how effectively they function as vectors for SRE.

A general function of SRE in herbivorous insect species might explain the presence of SRE in so many insects, as SRE are notorious producers of a variety of plant cell wall-degrading enzymes (PCWDEs) that are secreted to the extracellular environment (31). The notion of SRE as a functional component of the insect microbiome for the digestion of plant material is supported by the presence of SRE in the tested *D. floralis* laboratory rearing and a smaller, yet persistent, amount of SRE in most of the reared *P. xylostella* moths. However, the overall ratio of wild samples showing a low or no signal in the qPCR assay contradicts this assumption. In two general microbiome studies in Diptera from other ecological contexts, it was recently reported that some individuals carried large amounts of SRE, while other individuals of the same species with an otherwise comparable microbiome did not (32, 33).

The results from the field traps (Fig. 2), in combination with the findings from the laboratory-reared insects (Fig. 4), support the notion of a mutualistic relationship between multiple *Delia* species and SRE, as hypothesized in early work on *Delia* spp. (13, 16, 17). There, it was suggested by experiments with sterilized eggs that *D. platura* larvae needed SRE to survive and to develop normally under laboratory conditions. The relationship was therefore suggested to be specific or even symbiotic (13). In the case of the tested laboratory rearing of *D. floralis*, the last introduction of wild individuals to this was 5 years before testing. The results from the *D. floralis* samples therefore support the assumption that SRE are natural members of the microbiome of *Delia* species and are significantly more prevalent than in *P. xylostella* and *C. carnea*. Thus, it is likely that SRE and *Delia* species mutually add to their respective potentials to cause damage in their hosts. The relationships between SRE and specific insect groups might have various mutualistic facets. It was recently shown that a *Pectobacterium* sp. strain present in the *Delia radicum* gut microbiome was able to break down plant components that are toxic to the insect (34).

The detection of *D. solani* in insect samples from the Apelsvoll field (Table 1) suggests that some of the detected SRE originated from symptomatic plants in the tested fields. Sources of *D. solani* outside the field are highly unlikely, since this SRE species was not detected anywhere else in Norway that year, despite regular screening for it. Insects that take up large amounts of SRE from within the field might contribute to transmission between plants. However, being in vicinity to a symptomatic plant did not increase the proportion of positive samples in the traps in 2015 compared to 2016. More importantly, the traps set up in a field dedicated to the propagation of germfree minitubers contained a relatively high proportion of insects that tested positive compared to the other fields that year (Fig. 1, Overhalla). This suggests that there are inoculum sources outside the field or that SRE are part of the natural microbiome of some insects. The high proportion of individual specimens that tested positive at this field site shows the potential of insect-borne SRE to contribute to the initial infection of SRE-free plant material. Due to the high variety of identified insect species, this suggests a need to explore cultivation methods that minimize contact of the plants with insects to reduce initial SRE infection in seed production. Additionally, the detection of *Dickeya solani* in three insects suggests that insect trapping and bulk testing might be a tool that could be employed to monitor such quarantine pathogens that are commonly associated with insects.

Based on the results from this study, it is proposed that the SRE transmission in potato fields and other affected ecosystems is facilitated by a diverse range of potential vectors (Fig. 3). It seems that SRE have neither a low nor high vector specificity but rather represent a hybrid case, where the two mechanisms are acting simultaneously. While *D. platura* appeared to have the highest vector potential, other (*Delia*) species might be dominant depending on host plant prevalence, climatic conditions, and other factors impacting insect species composition. The background level of species carrying SRE with low or no vector potential, due to less likely acquisition and transmission scenarios, is suggested to be ubiquitous, as per the data shown in this work (Fig. 3). To test this, samples from different cultured and wild ecosystems under various climatic conditions need to be analyzed. Previous work suggests, for example, that *Drosophila* species act as a vector in other ecological contexts (18, 19), while they are nearly absent based on the data from this study (Table S2).

The work presented here suggests that the insect-borne SRE present in potato fields are more ubiquitous and heterogeneous than previously assumed. The results showed that at least 91 distinct insect species carried SRE in potato fields, including fields in which germfree tubers from tissue culture were propagated. This points to the potentially important role of a wide variety of insects in the ecology of SRE and may have implications for the initial infection of clean seed material and the currently employed control strategies for soft rot pathogens.

MATERIALS AND METHODS

Insect collection. Insects were collected using two to four yellow sticky traps in each of nine potato fields across the main potato-growing districts of Norway for two consecutive years (Fig. 1). In 2015, traps were set up next to potato plants with blackleg symptoms in six different fields for 6 to 10 days in summer (July to August). In 2016, three fields were sampled, including one field dedicated to the generation of prebasic 2 (P2; Norwegian seed certification) seeds from minitubers. That year, the traps were set up in a minimal distance of 10 m from any plant showing blackleg symptoms. Upon arrival at the Norwegian Institute of Bioeconomy Research, the traps were stored at -20°C .

One additional yellow sticky trap was set up adjacent to plants artificially inoculated with *Pectobacterium atrosepticum*, in Ås, Norway. Fully grown plants were inoculated by piercing the stem with a sterilized toothpick that was scraped over a bacterial lawn grown on LB agar plates (per liter, 10 g tryptone, 5 g yeast extract, 10 g NaCl, and 15 g agar). After lesions had developed at 10 days postinoculation, the trap was set out for 7 days in the beginning of August. From this trap, 64 insects were cut in half immediately after collection from the trap. One-half was used for qPCR testing with the PEC primer/probe set, while the other half was stored in 25% glycerol at -20°C before plating the bacteria.

DNA isolation. Insects were picked from the traps individually using xylene substitute (Sigma-Aldrich) to dissolve the glue of the traps (2015) or by careful removal without dissolving the glue (2016). While it was attempted to pick insects randomly off the traps, individuals were always included if the species appeared to occur ≤ 3 times on a trap and was distinguished by a marked phenotype. The number of tested insects per trap varied with the number of insects present on a given trap. The total DNA from each picked insect was isolated using the protocol recommended by the Canadian Centre for DNA Barcoding (35). The isolated DNA (50 μl per sample) was stored at -20°C .

qPCR for SRE detection. All individual insect samples were tested for the presence and quantity of SRE DNA using the PEC TaqMan assay, which amplifies a 119-bp sequence from SRE strains with high specificity (23). The reactions were conducted using 2 μl of DNA in a 20- μl reaction volume of SsoAdvanced universal probes supermix (Bio-Rad), in a CFX96 Touch real-time PCR detection system (Bio-Rad), with 3 min of initial denaturation at 95°C , followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Samples containing a large amount of SRE DNA (threshold set at $C_q < 28$) as determined by this qPCR analysis were used for species identification.

To find the relationship between the C_q signal and the number of CFU of SRE, a dilution series of *Pectobacterium polaris* strain NIBIO1006 (7) was tested. Three dilution series were produced from 3×1 ml of an overnight (o/n) culture of *P. polaris*, each grown from a single colony in LB broth at 28°C . Aliquots of undiluted culture and six 10-fold dilution steps until a 1:1,000,000 dilution for each of the three series were plated on LB medium. The colonies were counted after 48 h at room temperature for the two highest dilutions. The remaining dilutions were pelleted at $6,000 \times g$ for 10 min and resuspended in vertebrate lysis buffer (35), and the DNA was isolated as described for the insect samples (see above). The three dilution series were tested with the PEC primer/probe set, each in three qPCR replicates. According to these tests, the threshold C_q of 28 in the PEC assay corresponds to approximately 80,000 CFU of *P. polaris* for the protocol used in the insect experiments (Fig. S2).

Species identification of SRE. Specific TaqMan assays for *Dickeya solani*, *Pectobacterium atrosepticum*, *Pectobacterium parmentieri*, and *Pectobacterium carotovorum* subsp. *brasiliensis* were used to determine the species of the SRE present (Table 1). Due to large heterogeneity within *Pectobacterium carotovorum* subsp. *carotovorum*, no species-specific test is available. For the potato samples, SRE were isolated from the blackleg lesions of plants next to the traps collected in 2015. Eighteen diseased potato stems from six different fields with blackleg or stem rot symptoms were washed thoroughly under running water. Small pieces of tissue were then excised close to the border between healthy and diseased tissue and soaked in 0.5 ml sterile phosphate-buffered saline (PBS) for 30 min. The resulting extracts were streaked on modified Bulmer crystal violet pectate (MBCVP) plates (36) and incubated at two temperatures (room temperature and 37°C). After 48 h, bacterial colonies were picked from cavities indicating pectolytic activity and transferred to nutrient glucose agar plates (NGA; 23 g nutrient agar [Difco, USA], 5 g yeast extract, 10 g glucose, 1,000 ml distilled water) for growth at 25°C . All pectolytic isolates were initially identified by fatty acid methyl ester (FAME) analysis (37), and most of them were identified as either *Pectobacterium carotovorum* subsp. *carotovorum* or *Pectobacterium atrosepticum*. One isolate, identified by FAME as *Dickeya chrysanthemi* biovar V, was further analyzed with qPCR and species-specific primer/probe sets and was determined to be *Dickeya solani*.

All insect samples that were tested with the PEC assay and identified by sequencing were also tested with species-specific primer/probe sets for *D. solani*, *P. atrosepticum*, *P. carotovorum* subsp. *brasiliensis*, and *P. parmentieri* (Table 2). Real-time PCR was performed as described for the PEC assay, except for the assay for *P. carotovorum* subsp. *brasiliensis*, where the primer concentrations were adjusted as described in the original publication (38).

Sequencing of selected insect samples. DNA barcoding was done by PCR amplification of the mitochondrial cytochrome c oxidase subunit I (COI) from selected insect samples using the LCO1490/HCO2198 primer set (39). The PCR amplification was done as follows: 94°C denaturation (3 min), followed by 5 cycles of 94°C (30 s), 45°C (30 s), and 72°C (1 min), followed by 35 cycles of 94°C (30 s), 54°C (30 s), and 72°C (1 min), and a final elongation at 72°C for 10 min. The protocol was modified from the 2-step protocol for insect DNA barcoding (40). In a total reaction volume of 25 μl , 3 μl of 1:100-diluted DNA isolated from the insect samples was added. The COI amplicon was Sanger sequenced in both directions (GATC Biotech, Germany). The obtained sequences were trimmed in the 3' and 5' regions and the forward and reverse sequences assembled into a consensus. The consensus sequences were used for

TABLE 2 Primers and TaqMan probes used throughout this work

Target	Name	Primer or probe sequence (5'–3')			Reference
		Forward primer	Reverse primer	TaqMan probe	
All SRE	PEC	GTGCAAGCGTTAATCGGAATG	CTCTACAAGACTCTAGCCTGTCA GTTT	CTGGGCGTAAAGCGCACGCA	23
<i>Dickeya solani</i>	SOL-C	GCCTACACCATCAGGGCTAT	ACACTACAGCGCGCATAAAC	CCAGGCCGTGCTCGAAATCC	23
<i>Pectobacterium atrosepticum</i>	ECA	CGGCATCATAAAACACGCC	CCTGTGTAATATCCGAAAGGTGG	ACATTTCAGGCTGATATTCCCC CTGCC	23
<i>Pectobacterium parmentieri</i>	Pw	TCTGTTCATGTCAACGCAGGTA	AGGTAACCGCAATTGCTCAA	TGTGCGCAACCTG	38
<i>Pectobacterium carotovorum</i> subsp. <i>brasiliensis</i>	Pcbr	TGCGGGTCTGCGTTTC	TGGCGGTTTCGCAATAT	CAAGGCACGATACG	38
Insect COI barcode region	COI Folmer	GGTCAACAAATCATAAAGATA TTGG	TAAACTTCAGGGTGACCAAAA AATCA		39

species identification in the BOLD online interface for COI barcode identification with the Species Level Barcode Records database (41).

Accession number(s). All identified nucleotide sequences were deposited in the GenBank database under the accession numbers [MG673557](#) to [MG673923](#).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.00281-18>.

SUPPLEMENTAL FILE 1, PDF file, 1.1 MB.

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