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1 # Soft rot Enterobacteriaceae are carried by a large range of insect species in
2 # potato fields

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8

9 **Running Head:** Identifying insects carrying soft rot bacteria

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11

12 **Keywords:** plant pathology, soft rot, insect vectors, potato

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13 **Abstract:** Pathogenic soft rot Enterobacteriaceae (SRE) belonging to the genera
14 *Pectobacterium* and *Dickeya* cause diseases in potato and numerous other crops. Seed
15 potatoes are the most important source of infection, but how initially pathogen-free tubers
16 become infected remains an enigma. Since the 1920s, insects have been hypothesized to
17 contribute to SRE transmission. To validate this hypothesis and to map the insect species
18 potentially involved in SRE dispersal, we have analyzed the occurrence of SRE in insects
19 recovered from potato fields over a period of two years. Twenty-eight yellow sticky traps
20 were set up in ten potato fields throughout Norway to attract and trap insects. Total DNA
21 recovered from over 2000 randomly chosen trapped insects was tested for SRE, using a
22 specific qPCR TaqMan assay, and insects that tested positive were identified by DNA
23 barcoding. Although the occurrence of SRE-carrying insects varied, they were found in all the
24 tested fields. While *Delia* species were dominant among the insects that carried the highest
25 amount of SRE, more than 80 other SRE-carrying insect species were identified, in varying
26 numbers. Additionally, the occurrence of SRE in three laboratory-reared insect species was
27 analyzed, and this suggested that SRE are natural members of some insect microbiomes with
28 herbivorous *Delia floralis* carrying more SRE, compared to cabbage moth (*Plutella xylostella*)
29 and carnivorous green lacewing larvae (*Chrysoperla carnea*). In summary, the high
30 proportion, variety and ubiquity of insects that carried SRE shows the need to address this
31 source of the pathogens to reduce initial infection of seed material.

32

33 **Importance:** Soft rot Enterobacteriaceae are among the most important pathogens of a wide
34 range of vegetables and fruits. The bacteria cause severe rots in the field and in storage,
35 leading to considerable harvest losses. In potato, efforts to understand how soft rot bacteria

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36 **infect and spread between healthy plants have been made for over a century. Early on, fly**
37 **larvae were implicated in the transmission of these bacteria. This work aimed at**
38 **investigating the occurrence of soft rot bacteria in insects present in potato fields and**
39 **identifying the species of these insects to better understand the potential of this suspected**
40 **source of transmission. In all tested potato fields, a large proportion of insects were found to**
41 **carry soft rot bacteria. This suggests a need to give more weight to the role of insects in soft**
42 **rot ecology and epidemiology to design more effective pest management strategies that**
43 **integrate this factor.**

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44 **Introduction**

45 Soft rot *Enterobacteriaceae* (SRE) are pathogenic species of the genera *Pectobacterium* and
46 *Dickeya* that cause soft rots in plant species from 50 % of angiosperm plant orders, including
47 a wide variety of economically important crops such as potato, tomato, onion, pepper, and
48 cabbage (1). In potato, SRE cause soft rot in both tubers and stems. The bacteria enter
49 potato tubers through lenticels as well as fresh wounds on the tubers, roots and above-
50 ground parts of the plant (2). Blackleg symptoms follow soft rot in an infected seed tuber
51 piece, and a subsequent spreading of the pathogen through the vascular system (3).

52 The SRE species responsible for the most significant pre- and post-harvest losses in potato
53 are *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *brasiliensis*,
54 *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium parmentieri*, *Dickeya*
55 *dianthicola* and *Dickeya solani* (4-6). Some isolates of *Pectobacterium carotovorum* subsp.
56 *carotovorum* were recently re-classified into the new species *Pectobacterium polaris* (7).

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

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

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

81 In addition to the detailed work done on *Delia* and *Drosophila* species, studies of other
82 insects potentially involved in SRE transmission were previously attempted around two
83 potato waste dumps (9) and one field site for the propagation of clean seed material (20) in
84 Scotland. However, since these studies were conducted in the 1970's, detection of SRE
85 required enrichment on artificial medium and insect identification relied on morphological
86 taxonomy. Despite strong indications of insects as a source of initial SRE infection in the field
87 that was tested, the efforts were only moderately conclusive since the isolation of bacteria
88 was partially done from bulked insects and some that were not identified beyond their

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89 taxonomical order. The morphological identification of the few insect specimens found to
90 carry SRE in those studies showed, among others, *Leptocera* spp., *Scaptomyza* spp., *Scatopse*
91 spp. and *Delia* spp. as well as *Drosophila* spp. and unidentified Diptera (true fly) specimens
92 to have carried SRE (9, 20).

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

101 The potential contribution of insects to initial SRE infection of potato and other crops, as
102 well as to the dissemination within and between fields, remains unclear. The objective of
103 this study therefore, was to examine the presence of SRE in insects in multiple potato fields
104 by molecular methods suited for a sufficiently sensitive and efficient detection of pathogens
105 from individual insects and more accurate identification of insect species. The test sites
106 included fields where plants were symptomatic and one location where seed material was
107 propagated from clean tubers generated from tissue culture. The latter was particularly
108 informative to examine the potential of insect-borne SRE to contribute to initial infection.
109 The chosen scope was intended to reveal new potential insect vectors that were not found
110 in previous work and give an indication of the overall distribution of SRE over various insect

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111 species. Showing this distribution is a first step in the identification of possible vector
112 candidates for SRE, thereby allowing for appropriate control measures to be developed.

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113 **Results**

114 **A substantial proportion of insect samples contains SRE**

115 To assess the potential of insects to present a viable inoculum for SRE transmission,
116 individual insects sampled from potato fields in Norway using sticky traps (Fig. S1) were
117 examined for two consecutive years (Fig. 1).

118 The presence of SRE was tested by using a qPCR assay targeting all *Dickeya* and
119 *Pectobacterium* species (23). The threshold for a positive test (Cq < 28) was chosen
120 conservatively to only include insects with a high load of SRE and corresponded to between
121 10 000 and 100 000 colony-forming units (cfu), as determined by a dilutions series
122 experiment (Fig. S2). SRE were isolated from an insect that tested positive, and caused soft
123 rot symptoms when inoculated in SRE-free minitubers (Fig. S3).

124 Insects from all traps in all fields contained high amounts of SRE with percentages ranging
125 from 4 % to 39 % of insects from a given trap (Table S1). Out of 2122 tested insects in total,
126 19 % were positive for SRE with the chosen threshold. The overall percentage of insects that
127 tested positive varied between 15 % in 2015 and 23 % in 2016 (Fig. 1).

128

129 **A diverse group of insect species carries SRE**

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

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135 The families most commonly found to carry a high amount of SRE were *Anthomyiidae* with
136 46 %, and *Muscidae* with 14 % of the identified samples (Table S2). *Delia* was the dominant
137 genus among the samples that tested positive with 36% in 2015, 30 % in 2016 and 32 % in
138 total (Fig. 2A). The most prominent species was *D. platura*, making up 19 % of all positive
139 samples. The positive specimens collected from traps in the northernmost field (Overhalla)
140 were dominated by *Delia coarctata,* with only one individual being identified as *D. platura*,
141 whereas *D. coarctata* only tested positive in the other locations sporadically (Table S2). In
142 addition to Diptera, a number of Hemiptera (true bugs), mainly leafhoppers of the species
143 *Empoasca decipiens*, tested positive in both years.

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

148

149 **Dissemination of SRE by insects within and between fields**

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

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

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

164

165 **Abundance of insect species carrying SRE shows two extremes**

166 While our data indicates a large variety of insect species to be capable of carrying high
167 amounts of SRE, the number of individuals that tested positive differed widely between
168 species. Two extremes were observed in the identified species: an abundance of species
169 with few individuals that tested positive versus few species with a large number of
170 individuals that tested positive (Fig. 3). As the most extreme in the latter group, *D. platura*
171 alone represents a fifth of all identified individuals. Together with nine other species, it
172 makes up more than 50 % of the individuals shown to carry a high number of SRE. The
173 remaining individuals belong to at least 79 different species with eight or less individuals
174 observed over both years. For 50 species, only one individual tested positive over both years
175 (Fig. 3, Table S2).

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178 **Laboratory-reared *Delia floralis* contains high amounts of SRE**

179 To investigate the relationship between SRE and *Delia* spp. further, we tested individuals
180 from two generations of a long-term laboratory rearing of *D. floralis* (turnip root fly). Of
181 these, 66 % of 94 individuals tested positive for SRE using the rather conservative threshold
182 level of Ct 28 (Fig. 4). For comparison we tested two other laboratory-reared insect species,
183 *Plutella xylostella* (cabbage moth) because of its similar rearing conditions and carnivorous
184 *Chrysoperla carnea* (common green lacewing) larvae. The number of specimens positive for
185 SRE was significantly higher in *D. floralis* samples than in the other tested species as well as
186 the samples trapped in the fields (Fig. 4). For *P. xylostella*, 13 % of 94 of the specimens
187 tested positive for SRE, and for the *C. carnea* larvae, only one out of 40 specimens tested
188 positive for SRE. Furthermore, the average amount of SRE was significantly higher in *D.*
189 *floralis* specimens than in *P. xylostella* and *C. carnea*. Interestingly, adult individuals of both
190 *C. carnea* and the closely related *Chrysoperla lucasina* (one each) that feed on pollen and
191 nectar tested positively in the wild, trapped samples (Table S2).

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192 **Discussion**

193 SRE have a broad host plant spectrum and can be found in rotting lesions of wild and
194 cultivated plants (1), which might attract a variety of insects for egg deposition or feeding. It
195 is therefore reasonable to assume that a number of different insect species encounter SRE in
196 varying amounts depending on their behavior. The results shown in Fig. 2 support this
197 assumption. The results suggest that Diptera are more likely to acquire or have SRE as
198 members of their microbiome than other insects. However, the bias towards Diptera might
199 be inherent to the sampling method with yellow sticky traps mounted above ground due to
200 exclusion of ground-dwelling insect species and the color of the traps.

201 More than half of the identified insect species that tested positive for SRE were only
202 represented by one individual in both years (Fig. 3). Since only individuals that tested
203 positive were identified, it is not possible to infer the proportion of individuals of a given
204 species that was carrying SRE. Contamination of some individuals on the traps by aerosols or
205 cross-contamination from other insects that carried a lot of SRE cannot be excluded.
206 However, aerosol contamination would in principle be expected to be higher for samples
207 taken close to symptomatic plants, but the proportion of positive samples was comparable
208 (Table S1). Cross-contamination was assumed to be negligible since the sampled insects
209 rarely were in contact with each other on the traps. Likely explanations for species testing
210 positive in few individuals could be that these species were either not abundantly present at
211 the time and location of the experiment, not trapped, not tested, or simply are not
212 commonly associated with SRE. If they were not commonly associated, this would suggest
213 that at least some of the species that tested positive and were identified might not be
214 dedicated vectors for SRE. However, some degree of stochastic transmission from these

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215 individuals is conceivable, given a sufficient presence of insects carrying high amounts of the
216 pathogen. SRE have been shown to cause systemic infections upon inoculation in wounded
217 tubers, stems and leaves of potato under suitable conditions (25). This suggests a potential
218 mechanism for stochastic transmission of SRE by various insects that visit and cause plant
219 wounds. In such cases SRE could be applied to and transferred between wounds by insects
220 that retain the bacteria on their surface or mouth parts. Alternatively, SRE could be
221 introduced during wounding by insects that carry SRE internally for a short period.

222 *D. platura* stood out as the Dipteran species that carried SRE most frequently in both years
223 of the study. In addition, six other *Delia* species were frequent carriers of SRE, which
224 supports earlier work done on the relationship between SRE and various *Delia* species (13,
225 16, 17). The ecology of *Delia* species explains the acquisition of SRE at the larval stage, from
226 either rotten plant tissue or vertical transmission from the mother via the egg surface (13).
227 SRE infection of plants through *Delia* spp. has been shown from the larvae to the seed
228 material of their host (13, 16, 17), as well as from adult flies to wounded petioles and leaves
229 of potato plants (14). In addition to the transmission, long-term survival of SRE in the pupae
230 of *D. platura* that overwinter buried in the soil (26), offers a favorable means for the bacteria
231 to survive the winter in spite of prolonged freezing periods in temperate climates. Normally
232 SRE survive poorly in the environment in temperate climate (27).

233 In addition to Diptera, some specimens of the hemipteran leafhopper *E. decipiens* tested
234 positive for SRE. *E. decipiens* has been shown to transmit '*Candidatus* Phytoplasma asteris'
235 to daisies by feeding on leaves (28) and has previously been described as a potato pest (29).
236 Plant pests, like *E. decipiens,* are likely vector candidates since leafhoppers actively damage
237 the plant tissue by their stylet-like mouthparts that they use for sucking plant sap (30),

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238 thereby creating suitable conditions for SRE infection (25). Dedicated efforts to show
239 transmission of SRE to potato or other plants by the different insect species that were
240 identified here are needed to show how effectively they function as vectors for SRE.

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

251 The results from the field traps (Fig. 2) in combination with the findings from the laboratory-
252 reared insects (Fig. 4) support the notion of a mutualistic relationship between multiple
253 *Delia* species and SRE, as hypothesized in early work on *Delia* spp. (13, 16, 17). There, it was
254 suggested by experiments with sterilized eggs that *D. platura* larvae needed SRE to survive
255 and to develop normally under laboratory conditions. The relationship was therefore
256 suggested to be specific or even symbiotic (13). In the case of the tested laboratory rearing
257 of *D. floralis*, the last introduction of wild individuals to this was five years before testing.
258 The results from the *D. floralis* samples therefore support the assumption that SRE are
259 natural members of the microbiome of *Delia* species, and significantly more prevalent than
260 in *P. xylostella* and *C. carnea*. Thus, it is likely that SRE and *Delia* species mutually add to

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261 their respective potential to cause damage in their hosts. The relationships between SRE
262 and specific insect groups, might have various mutualistic facets. It was recently shown that
263 a *Pectobacterium* sp. strain present in the *Delia radicum* gut microbiome was able to break
264 down plant components that are toxic to the insect (34).

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

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

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

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302 **Materials and Methods**

303 Insect collection

304 Insects were collected using two to four yellow sticky traps in each of nine potato fields
305 across the main potato growing districts of Norway for two consecutive years (Fig. 1). In
306 2015, traps were set up next to potato plants with blackleg symptoms in six different fields
307 for 6-10 days in summer (July-August). In 2016, three fields were sampled, including one
308 field dedicated to the generation of P2 seeds from minitubers. That year, the traps were set
309 up in a minimal distance of 10 m to any plant showing blackleg symptoms. Upon arrival in
310 the institute, the traps were stored at -20 °C.

311 One additional yellow sticky traps was set up adjacent to plants artificially inoculated with
312 *Pectobacterium atrosepticum*, in Ås. Fully grown plants were inoculated by piercing the stem
313 with a sterilized toothpick that was scraped over a bacterial lawn grown on LB agar plates
314 (10 g tryptone, 5 g yeast extract, 10 g NaCl and 15 g agar for 1 L). After lesions had
315 developed 10 dpi, the trap was set out for 7 days in the beginning of August. From this trap,
316 64 insects were cut in half immediately after collection from the trap. One half was used for
317 qPCR testing with the PEC primer/probe set while the other half was stored in 25% glycerol
318 at -20 °C before plating of the bacteria.

319

320 DNA isolation

321 Insects were picked from the traps individually using Xylene Substitute (Sigma Aldrich) to
322 dissolve the glue of the traps (2015) or careful removal without dissolving the glue (2016).
323 While it was attempted to pick insects randomly off the traps, individuals were always

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324 included if the species appeared to occur three times or less on a trap and was distinguished
325 by a marked phenotype. The number of tested insects per trap varied with the number of
326 insects present on a given trap. The total DNA from each picked insect was isolated using the
327 protocol recommended by the Canadian Centre for DNA Barcoding (35). The isolated DNA
328 (50µL per sample) was stored at -20 °C.

329

330 qPCR for SRE detection

331 All individual insect samples were tested for the presence and quantity of SRE DNA using the
332 PEC TaqMan assay, which amplifies a 119 bp sequence from SRE strains with high specificity
333 (23). The reactions were conducted using 2 µL of DNA in 20 µL reaction volume of
334 SsoAdvanced™ Universal Probes Supermix (Bio-Rad), in a CFX96 Touch™ Real-Time PCR
335 Detection System (Bio-Rad), with 3 min of initial denaturation at 95 °C followed by 40 cycles
336 with 95 °C for 10 sec and 60 °C for 30 sec. Samples containing a high amount of SRE DNA
337 (threshold set at Cq <28) as determined by this qPCR analysis were used for species
338 identification.

339 To find the relationship between the Cq signal and the number of colony forming units (cfu)
340 of SRE a dilution series of *Pectobacterium polaris* strain NIBIO1006 (7) was tested. Three
341 dilution series were produced from 3x 1 mL of an o/n culture of *P. polaris*, each grown from
342 a single colony in LB broth at 28 °C. Aliquots of undiluted culture and six 10-fold dilution
343 steps until 1: 1 000 000 for each of the three series were plated on LB medium. The colonies
344 were counted after 48 h at room temperature for the two highest dilutions. The remaining
345 dilutions were pelleted at 6000 *g* for 10 min, resuspended in vertebrate lysis buffer (35) and
346 the DNA was isolated as described for the insect samples (see above). The three dilution

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347 series were tested with the PEC primer/probe set, each in three qPCR replicates. According
348 to these tests, the threshold Cq of 28 in the PEC assay corresponds to approximately 80 000
349 cfu of *P. polaris* for the protocol used in the insect experiments (Fig. S2).

350

351 **Species identification of SRE**

352 For DNA samples from insect specimens, specific TaqMan assays for *Dickeya solani*,
353 *Pectobacterium atrosepticum*, *Pectobacterium parmentieri* and *Pectobacterium carotovorum*
354 subsp. *brasiliensis* were used to determine the species of the SRE present (Table 1). Due to
355 large heterogeneity within the species *Pectobacterium carotovorum* subsp. *carotovorum*, no
356 species specific test is available. For the potato samples, SRE were isolated from the blackleg
357 lesions of plants next to the traps collected in 2015. Eighteen diseased potato stems from six
358 different fields with blackleg or stem rot symptoms were washed thoroughly under running
359 water. Small pieces of tissue were then excised close to the border between healthy and
360 diseased tissue and soaked in 0.5 ml sterile phosphate buffered saline (PBS) for 30 minutes.
361 The resulting extracts were streaked on MBCVP plates (36), and incubated at two
362 temperatures (room temperature, 37 °C). After 48 h, bacterial colonies were picked from
363 cavities indicating pectolytic activity, and transferred to nutrient glucose agar plates (NGA;
364 23 g nutrient agar (Difco, USA), 5 g yeast extract, 10 g glucose, 1000 ml distilled water) for
365 growth at 25 °C. All pectolytic isolates were initially identified by fatty acid methyl ester
366 (FAME) analysis (37), most of them were identified as either *Pectobacterium carotovorum*
367 subsp. *carotovorum* or *Pectobacterium atrosepticum*. One isolate, identified by FAME as
368 *Dickeya chrysanthemi* biovar V was further analyzed with qPCR and species specific
369 primer/probe sets and proved to be *Dickeya solani*.

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

375

376 Sequencing of selected insect samples

377 DNA barcoding was done by PCR amplification of the mitochondrial cytochrome c oxidase
378 subunit I (COI) from selected insect samples using the LCO1490/HCO2198 primer set (39).
379 The PCR amplification was done as follows: 94 °C denaturation (3 min), followed by 5 cycles
380 of 94 °C (30 sec), 45 °C (30 sec), 72 °C (1 min) followed by 35 cycles with 94 °C (30 sec), 54 °C
381 (30 sec), 72 °C (1 min) and a final elongation at 72 °C for 10 min. The protocol was modified
382 from the 2 step protocol for insect DNA barcoding (40). In a total reaction volume of 25 µL, 3
383 µl of 1:100 diluted DNA isolated from the insect samples was added. The COI amplicon was
384 Sanger sequenced in both directions (GATC Biotech, Germany). The obtained sequences
385 were trimmed in the 3' and 5' regions and the forward and reverse sequences assembled
386 into a consensus. The consensus sequences were used for species identification in the BOLD
387 online interface for COI barcode identification with the Species Level Barcode Records
388 database (41). All identified nucleotide sequences are deposited in the GenBank database
389 under the accession numbers MG673557 - MG673923.

390

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397

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511 **Table 1.** SRE species detected in symptomatic plants, and insects from potato fields. Identification of SRE was done by using species specific
512 TaqMan assays on insects that tested positive in the PEC assay, or by FAME analysis of isolates from blackleg lesions of potato plants adjacent
513 to traps. The numbers refer to insect specimens that tested positive for each of the species specific TaqMan assays, for each field. The number
514 of insects that tested positive for SRE with the general PEC TaqMan assay in each field is given under 'PEC Cq < 28'. The SRE species that were
515 isolated from the symptomatic potato plants adjacent to the traps are indicated as 'found' (+) and 'not found' (-) based on the FAME
516 identification. The insect data for *Pectobacterium carotovorum* subsp. *carotovorum* is not available ('N/A') since there was no specific TaqMan
517 assay for it. In the 2016 fields, symptomatic potato plants were not tested.

Field Year	Source	*Dickeya solani*	*Pectobacterium atrosepticum*	*Pectobacterium carotovorum* subsp. *brasiliensis*	*Pectobacterium carotovorum* subsp. *carotovorum*	*Pectobacterium parmentieri*	PEC Cq < 28*
Apelsvoll	Insects	3	6	0	N/A	2	38
2015	Potato plants	+	+	-	-	-	
Brandval	Insects	0	6	0	N/A	2	26
2015	Potato plants	-	+	-	-	-	
Gjervoldstad	Insects	0	24	0	N/A	2	45
2015	Potato plants	-	+	-	-	-	
Hamar	Insects	0	11	0	N/A	1	43
2015	Potato plants	-	+	-	+	-	
Larvik	Insects	0	0	0	N/A	1	8
2015	Potato plants	-	+	-	-	-	
Rygge	Insects	0	1	0	N/A	0	11

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2015	**Potato plants**	**-**	**+**	**-**	**+**	**-**	
Hamar 2016	**Insects**	**0**	**0**	**0**	**N/A**	**5**	**40**
Overhalla 2016	**Insects**	**0**	**13**	**0**	**N/A**	**34**	**103**
Reddal 2016	**Insects**	**0**	**27**	**0**	**N/A**	**10**	**62**
Ås 2016	**Insects**	**0**	**0**	**0**	**N/A**	**3**	**25**

518 *Out of 401 positive insects, ten were not tested with species specific TaqMan assays due to a limited amount of DNA.

519

520 **Table 2.** Primers and TaqMan probes used throughout this work.

Target	Name	Forward sequence (5'-3')	Reverse sequence (5'-3')	TaqMan probe (5'-3')	Reference
All SRE	PEC	GTGCAAGCGTTAATCGGAATG	CTCTACAAGACTCTAGCCTGTCAGTTTT	CTGGGCGTAAAGCGCACGCA	(23)
Dickeya solani	SOL-C	GCCTACACCATCAGGGCTAT	ACACTACAGCGCGCATAAAC	CCAGGCCGTGCTCGAAATCC	(23)
Pectobacterium atrosepticum	ECA	CGGCATCATAAAAACACGCC	CCTGTGTAATATCCGAAAGGTGG	ACATTCAGGCTGATATTCCCCCTGCC	(23)
Pectobacterium parmentieri	Pw	TCTGTTCAATGTCAACGCAGGTA	AGGTAACCGCAATTTGCTCAA	TGTGCGCAACCTG	(38)
Pectobacterium carotovorum subsp. *brasiliensis*	Pcbr	TGCGGGTTCTGCGTTTC	TGGCGCGTTCGCAATAT	CAAGGCACGATACG	(38)
Insect COI barcode region	COI Folmer	GGTCAACAAATCATAAAGATATTGG	TAAACTTCAGGGTGACCAAAAAATCA		(39)

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521 **Fig. 1.** Map of Norway with indicators for all field locations in 2015 (o) and 2016 (x) and
522 associated proportions of samples that tested positive (blue) and negative (grey) for SRE
523 using the PEC assay. Names of the field locations are given above each pie chart. Overall
524 proportions for 2015 (top), 2016 (center), and in total (bottom) are given on the right.
525 Distances of the traps from any symptomatic plants are indicated under each pie chart.
526 Fields with traps set up in a minimum distance of 10 m from any symptomatic plant did not
527 necessarily contain symptomatic plants. Further details are given in Table S1. (Map
528 templates were from Geonorge.)

529 **Fig. 2.** Identification, classification and proportions of insect specimens that tested positive
530 for SRE. (A) Genera of insect specimen that tested positive over both years (inner circle), as
531 well as species that tested positive in 2015 (second circle), 2016 (third circle) and over both
532 years (outer circle). Only taxa with more than 10 representatives over both years are shown,
533 the rest is represented as "other". (B) Insect families and orders for specimen that tested
534 positive over both years with the most prevalent family (Anthomyiidae) order (Diptera)
535 highlighted in black, and others in light grey.

536 **Fig. 3.** The relationship between the number of insect species and number of individuals for
537 a given species. 'Number of individuals' (X-axis) refers to the number of instances of a
538 species being identified while 'number of identified species' (Y-axis) refers to the number of
539 instances where one species was identified with the corresponding amount of individuals (X-
540 axis). Samples for 2015 (o), 2016 (x) and in total (filled circles) are shown.

541 **Fig. 4.** Number of SRE bacteria in insect samples from traps and from laboratory rearings
542 with median (black line) and distribution of all samples. The cfu was calculated using a linear
543 approximation for the relationship between Cq and cfu values from the dilution series data

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544 (Fig. S2); samples from $1:10^3$ to $1:10^6$ were used to create the linear approximation. For *Delia*
545 *floralis* 94 samples of adult flies from two consecutive generations were tested (47 each), for
546 *Plutella xylostella* 94 samples of adult moths were tested and for *Chrysoperla carnea* 40
547 samples of larvae were tested. The red line indicates the calculated cfu corresponding to the
548 Cq = 28 threshold used in the field samples. Letters a-d indicate significantly different
549 groups of samples according to Mann-Whitney test ($p < 0.05$); all combinations were tested
550 for both, Cq and calculated cfu values.

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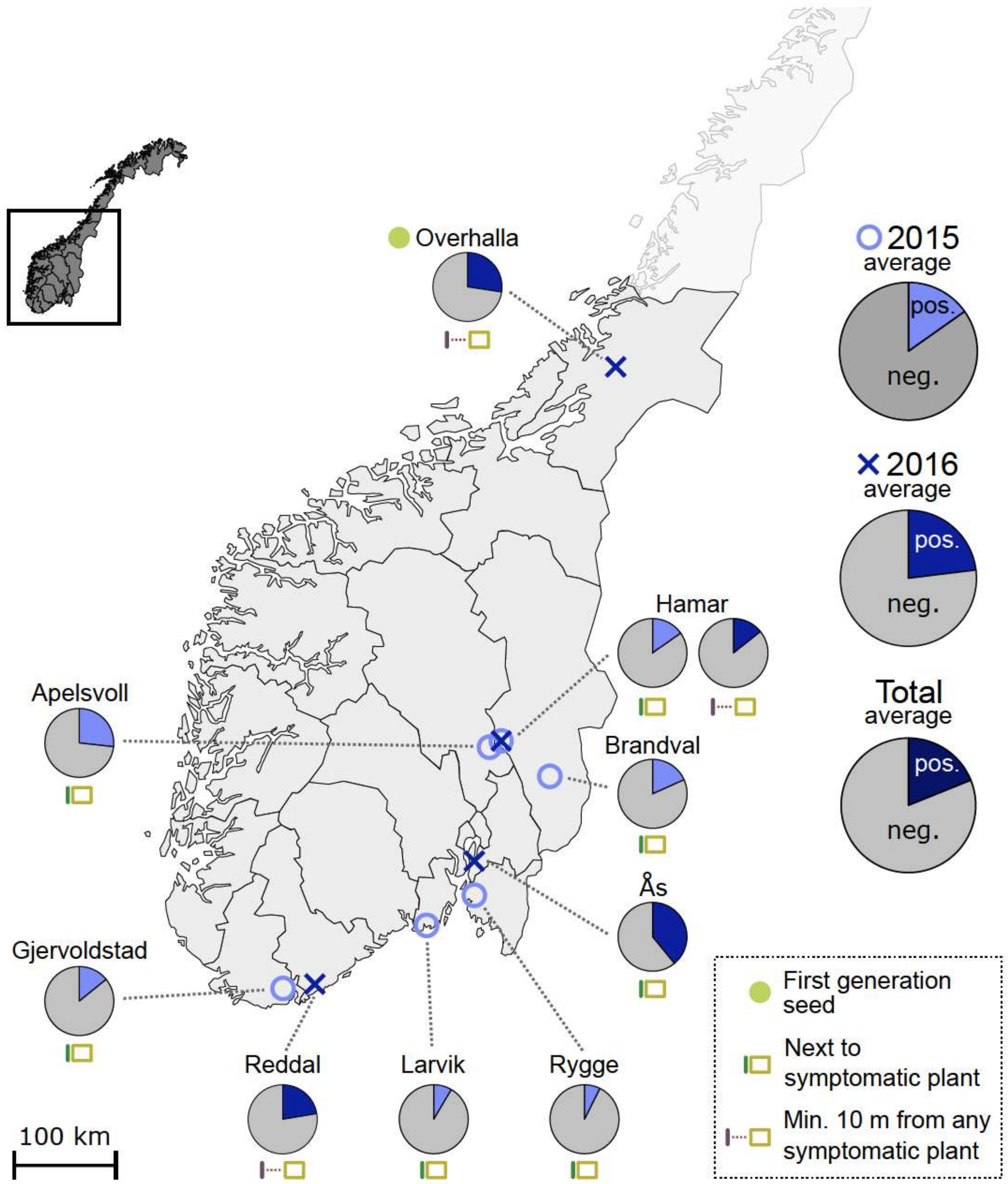

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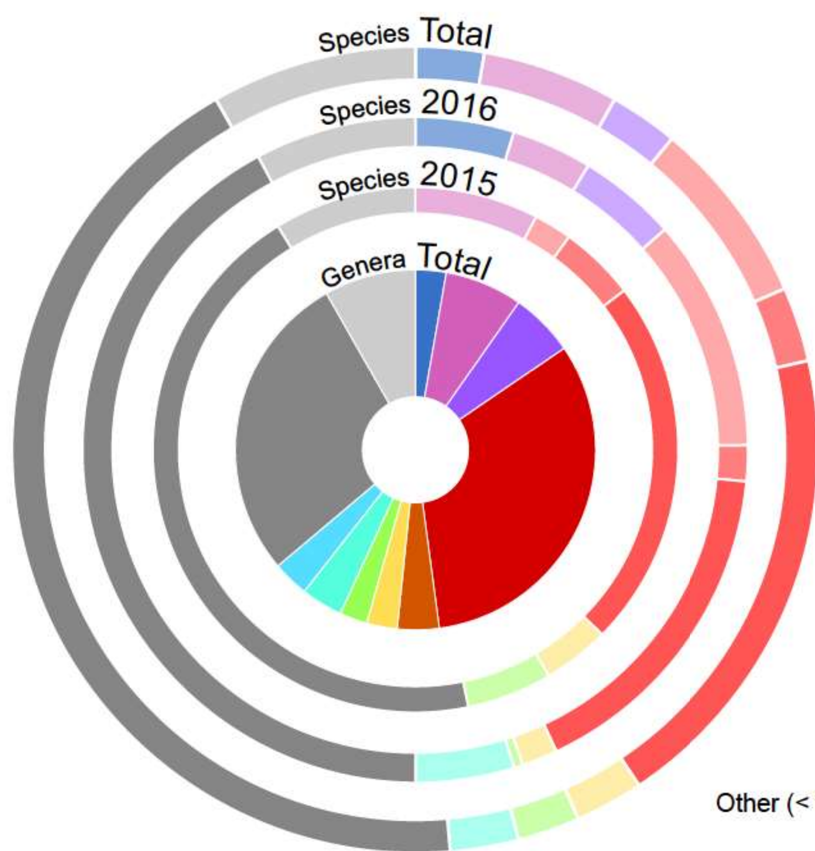

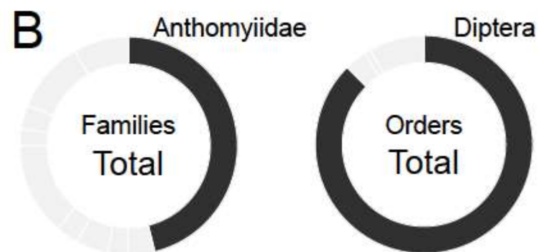

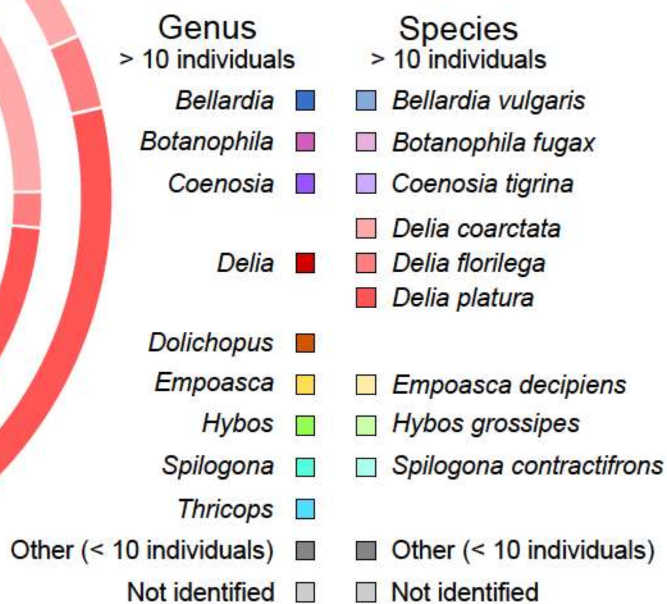

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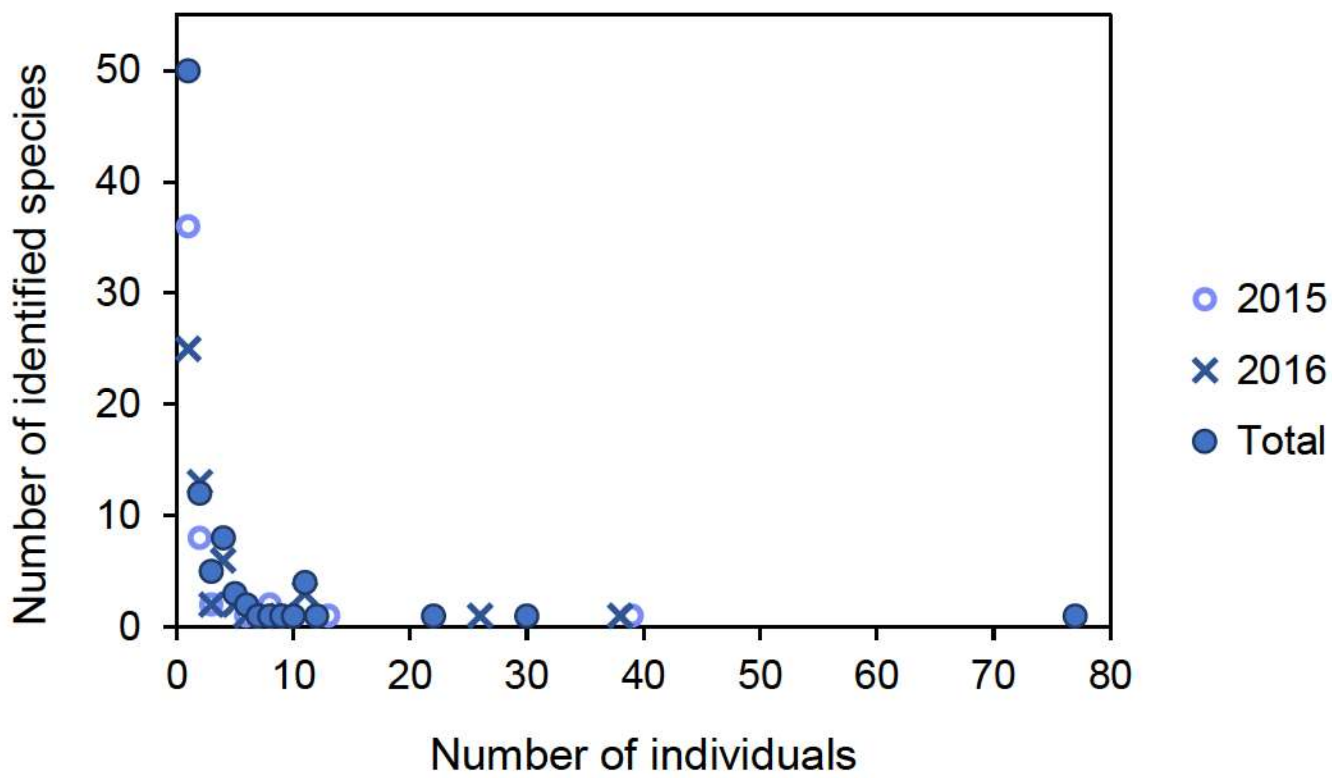

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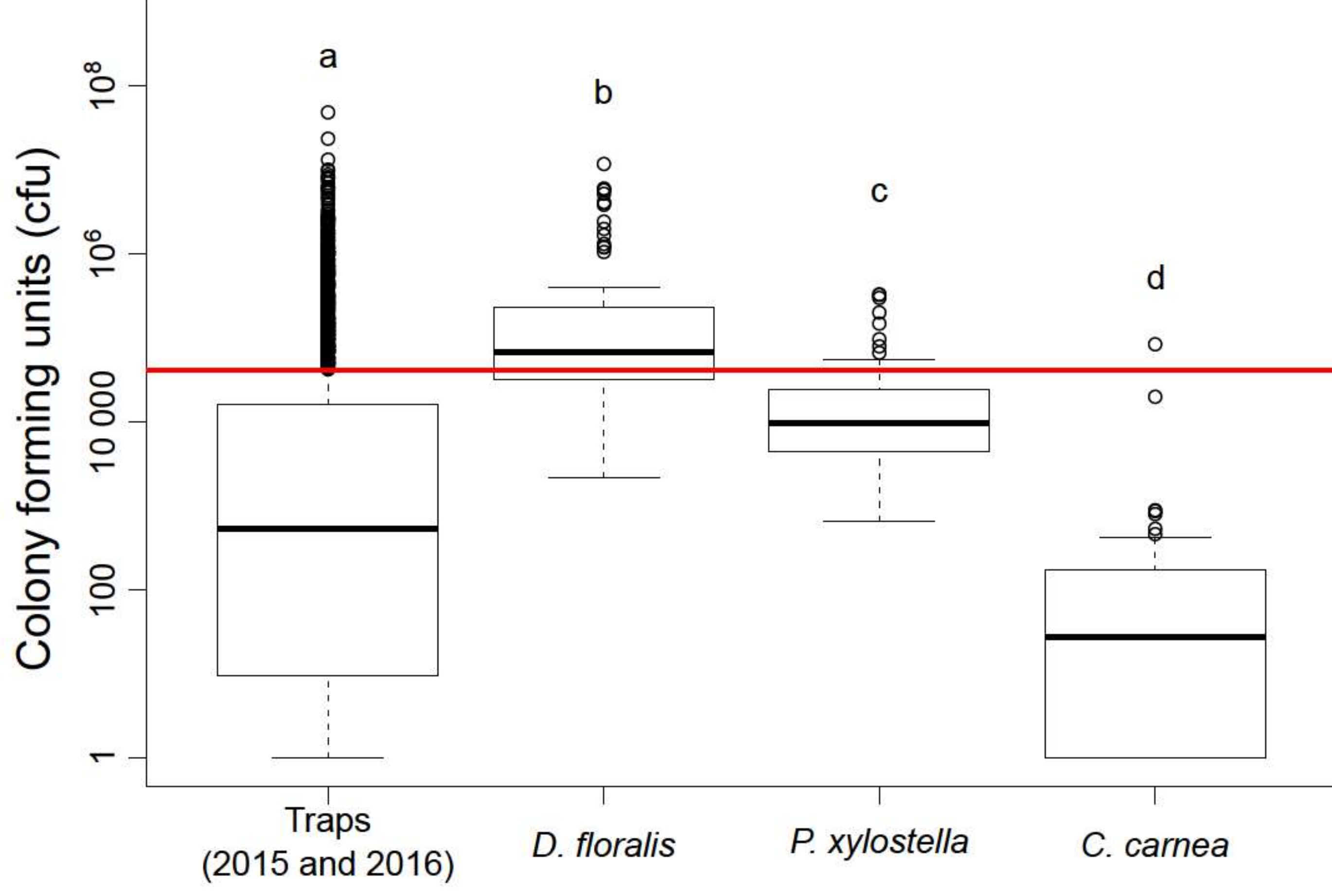

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Supplemental material

Soft rot Enterobacteriaceae are carried by a large range of insect species in potato fields

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Figures S1-S3

Tables S1-S2

Supplementary methods: Insect plating and pathogenicity testing; Insect rearings

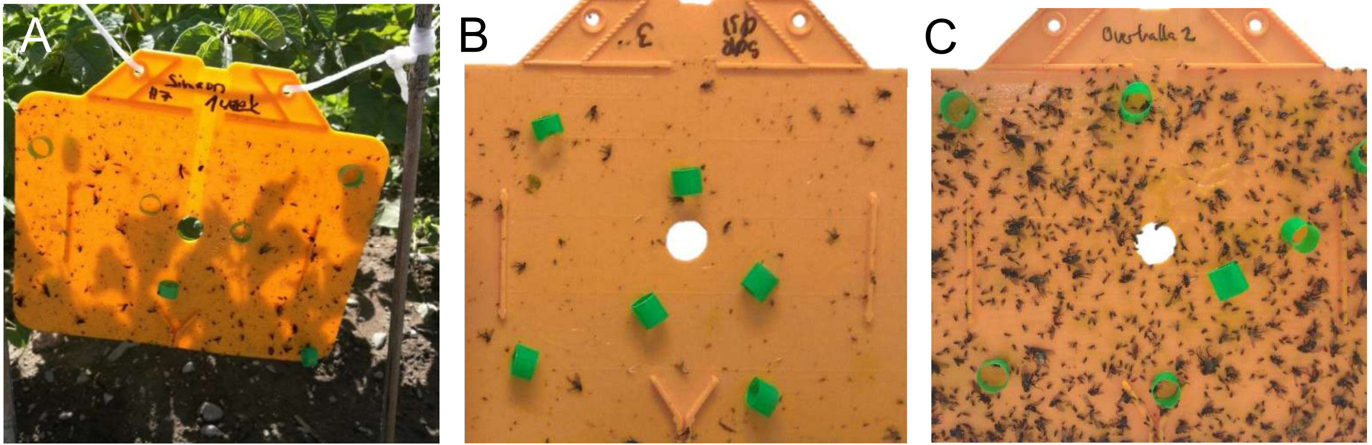

Fig. S1. Yellow sticky traps before processing. All traps are shown after having been in various fields for approximately one week. (A) Trap on field in Ås. (B) Trap number three from Rygge, collected in 2015 (Rygge 3) with relatively few insects on it. (C) Trap number two from the Overhalla field for the propagation of minitubers in 2016 (Overhalla 2). The pictures shown are representative for the amount of insects found in the respective location. The pictures were chosen because they represent two locations with a relatively low (B) and high (C) amount of trapped insects.

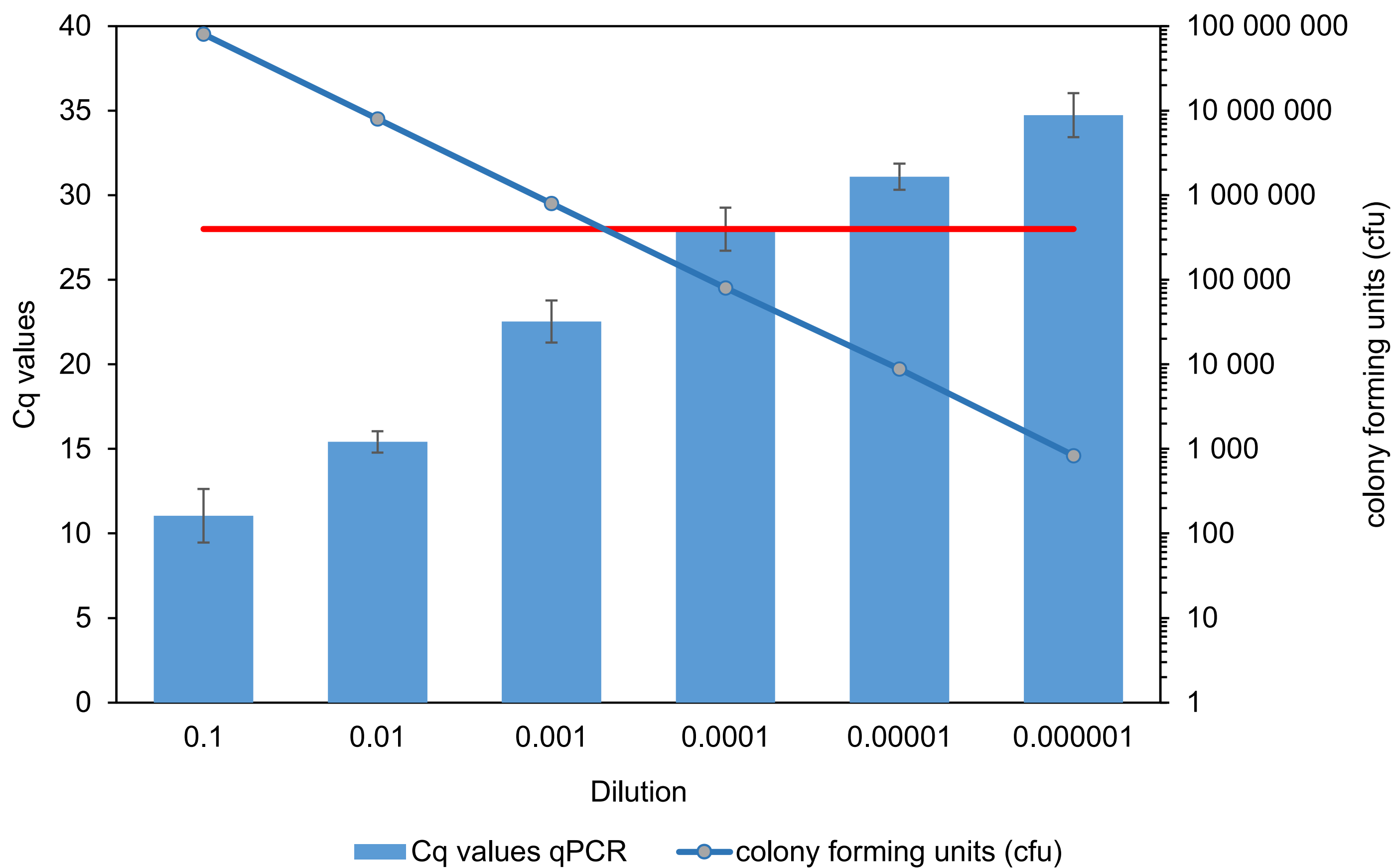

Fig. S2. Relationship between the Cq values obtained in the PEC qPCR assay and number of cfu after plating in a dilution series of *P. polaris* (strain NIBIO1006) on LB agar-plates. The bars show the average Cq values obtained in the PEC qPCR assays from three dilution series, each tested in three PCR replicates and their standard deviations. The line graph shows average cfu after plating from each of the three dilution series used for DNA isolation and qPCR. Cfu values were adjusted to match the volume used in the DNA isolation (50 µL). The red line indicates Cq = 28 which was used as a cut off to define insect samples with high SRE content.

Fig. S3. Rot progression in minitubers (cv. Asterix) after inoculation with SRE from a trapped insect, after 4 days of vacuum incubation. (A) Toothpick inoculation of one colony from plating of insect suspension (half an insect that tested positive for PEC in glycerol). (B) Positive control produced by scraping a sterile toothpick over an MBCVP plate after plating of *Pectobacterium polaris* (strain NIBIO1006). (C) Negative control produced by scraping a sterile toothpick over an MBCVP plate after plating of 25% glycerol and incubation as done for the other samples.

Table S1: Results of the qPCR detection of SRE DNA in insects for all traps in 2015 (traps next to symptomatic plants) and 2016 (traps min. 10 m from any symptomatic plants, except for the trap at Ås). For each trap location, the number of tested insects, the number of insects with a Cq < 28 in the qPCR assay, as well as the percentage of positively tested insects are shown.

Trap location	Year	Tested	Cq < 28	%	Closest symptomatic plant
Apelsvoll 1	2015	48	16	33.3	< 1 m
Apelsvoll 2	2015	46	9	19.6	< 1 m
Apelsvoll 3	2015	48	13	27.1	< 1 m
Apelsvoll total	**2015**	**142**	**38**	**26.8**	
Brandval 1	2015	52	4	7.7	< 1 m
Brandval 2	2015	42	14	33.3	< 1 m
Brandval 3	2015	48	8	16.7	< 1 m
Brandval total	**2015**	**142**	**26**	**18.3**	
Gjervoldstad 1	2015	127	22	17.3	< 1 m
Gjervoldstad 2	2015	94	18	19.1	< 1 m
Gjervoldstad 3	2015	96	5	5.2	< 1 m
Gjervoldstad total	**2015**	**317**	**45**	**14.2**	
Hamar 1	2015	46	11	23.9	< 1 m
Hamar 2	2015	126	18	14.3	< 1 m
Hamar 3	2015	110	14	12.7	< 1 m
Hamar total	**2015**	**282**	**43**	**15.2**	
Larvik 1	2015	48	4	8.3	< 1 m
Larvik 2	2015	46	4	8.7	< 1 m
Larvik total	**2015**	**94**	**8**	**8.5**	
Rygge 1	2015	47	5	10.6	< 1 m
Rygge 2	2015	47	2	4.3	< 1 m
Rygge 3	2015	54	4	7.1	< 1 m
Rygge total	**2015**	**148**	**11**	**7.4**	
Hamar 1	2016	96	16	16.7	> 10 m
Hamar 2	2016	92	9	9.8	> 10 m
Hamar 3	2016	92	15	16.3	> 10 m
Hamar total	**2016**	**280**	**40**	**14.3**	
Overhalla 1	2016	94	24	25.5	> 10 m
Overhalla 2	2016	94	20	21.3	> 10 m
Overhalla 3	2016	94	36	38.3	> 10 m
Overhalla 4	2016	94	23	24.5	> 10 m
Overhalla total	**2016**	**376**	**103**	**27.4**	
Reddal 1	2016	92	17	18.5	> 10 m
Reddal 2	2016	93	32	34.4	> 10 m
Reddal 3	2016	92	13	14.1	> 10 m
Reddal total	**2016**	**277**	**62**	**22.4**	
Ås (stem inoculation)	2016	64	25	39.1	< 1 m
Total 2015		**1125**	**171**	**15.2**	
Total 2016		**997**	**230**	**23.1**	
Total (both years)		**2122**	**401**	**18.9**	

Table S2: Numbers of insects found to contain a high amount of SRE, based on PEC TaqMan assay (Cq < 28), and their respective order, family, genus and species as identified by DNA barcoding.

Order				Family				Genus				Species				Fields									
	2015	2016	Total		2015	2016	Total		2015	2016	Total		2015	2016	Total	Ap 15	Br 15	Gj 15	Ha 15	La 15	Ry 15	Aa 16	Ha 16	Ov 16	Re 16
Diptera	147	204	351	Agromyzidae	0	2	2	*Chromatomyia*	0	2	2	*Chromatomyia fuscula*	0	2	2									2	
				Anthomyiidae	86	99	185	*Adia*	0	1	1	*Adia cinerella*	0	1	1								1		
								Alliopsis	1	0	1	*Alliopsis silvestris*	1	0	1			1							
								Botanophila	14	14	28	*Botanophila betarum*	0	4	4									4	
												Botanophila bicilaris	0	1	1							1			
												Botanophila fugax	13	9	22	5	3		5				1	5	3
												Botanophila gnava	1	0	1			1							
								Delia	61	69	130	*Delia coarctata*	4	26	30			4						26	
												Delia floralis	1	0	1			1							
												Delia florilega	8	4	12			4	3	1		2			2
												Delia nuda	0	1	1									1	
												Delia platura	39	38	77	2		10	23		4	1	10	1	26
												Delia quadripila	1	0	1			1							
												Delia radicum	8	0	8	1		4	1		2				
								Heterostylodes	1	3	4	*Heterostylodes sp.*	1	3	4			1					2	1	
								Hydrophoria	1	0	1	*Hydrophoria lancifer*	1	0	1			1							
								Lasiomma	2	2	4	*Lasiomma picipes*	2	2	4	1		1				1		1	
								Pegomya	0	5	5	*Pegomya solennis*	0	5	5								1	2	2
								Pegoplata	2	4	6	*Pegoplata aestiva*	2	2	4	1			1				1	1	
												Pegoplata infirma	0	2	2									2	
								Phorbia	4	1	5	*Phorbia genitalis*	4	1	5					4		1			
				Calliphoridae	4	12	16	*Bellardia*	0	11	11	*Bellardia vulgaris*	0	11	11									11	
								Melinda	3	0	3	*Melinda gentilis*	2	0	2	2									
												Melinda viridicyanea	1	0	1	1									
								Pollenia	1	1	2	*Pollenia pediculata*	1	0	1	1									
												Pollenia rudis	0	1	1							1			
				Chironomidae	1	1	2	*Nanocladius*	0	1	1	*Nanocladius dichromus*	0	1	1									1	
								Smittia	1	0	1	*Smittia* sp.	1	0	1						1				
				Dolichopodidae	6	13	19	*Chrysotus*	2	1	3	*Chrysotus cilipes*	1	1	2						1				1
												Chrysotus femoratus	1	0	1		1								
								Dolichopus	3	12	15	*Dolichopus cilifemoratus*	1	0	1						1				
												Dolichopus longicornis	2	2	4		1		1						2
												Dolichopus plumipes	0	6	6							2	1	2	1
												Dolichopus simplex	0	4	4									4	
								Sciapus	1	0	1	*Sciapus spiniger*	1	0	1			1							
				Drosophilidae	5	3	8	*Drosophila*	3	2	5	*Drosophila* sp. GS01	3	2	5	1	1	1					1		1
								Scaptomyza	2	1	3	*Scaptomyza flava*	1	0	1			1							
												Scaptomyza griseola	0	1	1								1		
												Scaptomyza pallida	1	0	1			1							
				Fanniidae	2	3	5	*Fannia*	2	3	5	*Fannia armata*	0	1	1							1			
												Fannia canicularis	1	0	1					1					
												Fannia polycheta	1	1	2						1			1	
												Fannia sociella	0	1	1									1	
				Hybotidae	10	4	14	*Hybos*	9	1	10	*Hybos grossipes*	9	1	10	7			2			1			
								Platypalpus	1	3	4	*Platypalpus interstinctus*	1	3	4		1								3

				Lauxaniidae	1	4	5	*Calliopum*	1	2	3	*Calliopum aeneum*	1	2	3		1							2	
								Meiosimyza	0	2	2	*Meiosimyza illota*	0	2	2								1	1	
				Limoniidae	1	0	1	*Dicranomyia*	1	0	1	*Dicranomyia frontalis*	1	0	1		1								
				Muscidae	7	48	55	*Azelia*	0	1	1	*Azelia cilipes*	0	1	1									1	
								Coenosia	4	19	23	*Coenosia mollicula*	0	2	2									2	
												Coenosia pumila	2	5	7		2					5			
												Coenosia rufipalpis	2	1	3		2								1
												Coenosia tigrina	0	11	11										11
								Hebecnema	1	0	1	*Hebecnema vespertina*	1	0	1		1								
								Helina	0	1	1	*Helina reversio*	0	1	1							1			
								Muscina	1	0	1	*Muscina levida*	1	0	1	1									
								Spilogona	0	15	15	*Spilogona contractifrons*	0	11	11									11	
												Spilogona pacifica	0	4	4									4	
								Thricops	1	12	13	*Thricops cunctans*	0	4	4							1		3	
												Thricops innocuus	1	8	9			1					4	4	
				Pallopteridae	1	0	1	*Palloptera*	1	0	1	*Palloptera ustulata*	1	0	1					1					
				Phoridae	2	0	2	*Diplonevra*	1	0	1	*Diplonevra freyi*	1	0	1				1						
								Megaselia	1	0	1	*Megaselia ciliata*	1	0	1			1							
				Sarcophagidae	2	0	2	*Sarcophaga*	2	0	2	*Sarcophaga pumila*	2	0	2			1	1						
				Scathophagidae	1	0	1	*Norellisoma*	1	0	1	*Norellisoma spinimanum*	1	0	1			1							
				Sciaridae	4	6	10	*Bradysia*	0	4	4	*Bradysia praecox*	0	2	2									2	
												Bradysia sp.	0	2	2								2		
								Ctenosciara	3	0	3	*Ctenosciara hyalipennis*	3	0	3		3								
								Hyperlasion	1	0	1	*Hyperlasion wasmanni*	1	0	1			1							
								Lycoriella	0	2	2	*Lycoriella sativae*	0	2	2								2		
				Sciomyzidae	1	0	1	*Euthycera*	1	0	1	*Euthycera fumigata*	1	0	1				1						
				Sepsidae	0	3	3	*Themira*	0	3	3	*Themira annulipes*	0	3	3										3
				Simuliidae	1	0	1	*Simulium*	1	0	1	*Simulium reptans*	1	0	1		1								
				Sphaeroceridae	0	1	1	*Pseudocollinella*	0	1	1	*Pseudocollinella humida*	0	1	1										1
				Syrphidae	11	3	14	*Cheilosia*	0	1	1	*Cheilosia ruficollis*	0	1	1							1			
								Episyrphus	6	0	6	*Episyrphus balteatus*	6	0	6	5			1						
								Melanostoma	4	0	4	*Melanostoma mellinum*	2	0	2	1	1								
												Melanostoma scalare	1	0	1	1									
												Melanostoma sp.	1	0	1					1					
								Platycheirus	1	0	1	*Platycheirus clypeatus*	1	0	1		1								
								Syrphus	0	2	2	*Syrphus ribesii*	0	1	1								1		
												Syrphus vitripennis	0	1	1								1		
				Tachinidae	1	2	3	*Eriothrix*	0	1	1	*Eriothrix rufomaculata*	0	1	1									1	
								Medina	1	0	1	*Medina luctuosa*	1	0	1		1								
								Voria	0	1	1	*Voria ruralis*	0	1	1							1			
Hemiptera	8	4	12	Cicadellidae	8	4	12	*Empoasca*	7	4	11	*Empoasca decipiens*	7	4	11	7							4		
								Macrosteles	1	0	1	*Macrosteles laevis*	1	0	1		1								
Hymenoptera	0	2	2	Ichneumonidae	0	2	2	*Diadegma*	0	1	1	*Diadegma fenestrale*	0	1	1										1
								Sussaba	0	1	1	*Sussaba dorsalis*	0	1	1							1			
Neuroptera	1	1	2	Chrysopidae	1	1	2	*Chrysoperla*	1	1	2	*Chrysoperla carnea*	0	1	1										1
												Chrysoperla lucasina	1	0	1				1						
Not identified	15	19	34	Not identified	15	19	34	Not identified	15	19	34	Not identified	15	19	34	1	4	7	2	0	1	4	6	6	3
Total	171	230	401	Total	171	230	401	Total	171	230	401	Total	171	230	401	38	26	45	43	8	11	25	40	103	62

Supplementary methods

Insect plating and pathogenicity testing

Insect bodies in glycerol solution with high SRE content, as defined by the PEC TaqMan assay (Cq < 28), were plated on MBCVP medium (1). Glycerol solution from a sample that showed no signal or a low SRE content were plated for comparison. The plates were incubated at room temperature and cavity formation was evaluated 4 days after plating. For pathogenicity assessment, colonies were scraped from cavities using sterile toothpicks, and these were pierced into minitubers at the stolon end and then broken off to create a smooth surface. The pierced minitubers were incubated in vacuum using suitable plastic bags at room temperature for 4 days, each bag containing three tubers as biological replicates. After opening the bags, the tubers were cut in half and rot formation documented in pictures.

Insect rearings

Delia floralis were supplied from a rearing at NIBIO. The original stock material was collected as pupae from commercial vegetable fields in the beginning of the 1990s, with occasional additions at roughly 5 year intervals. The newest addition to the rearing was in 2012. The flies were reared in cages in a climate-controlled room with the parameters: day/night 16/8 h, constant temperature 18 °C, RH 70 %. After hatching from pupae and mating, the flies were presented an oviposition substrate consisting of a piece of rutabaga (*Brassica napus* var. *napobrassica*) on sand in a Petri dish. Eggs were transferred from the substrate to a larger piece of rutabaga in one-half litre of sand for the larval development. The larvae tunnelled into the sand for pupation and hatched as adults after approximately 22 days. Adults were given water through a wick in a beaker and food to facilitate egg development in the females (4:1 Brewer's yeast:glucose). Individuals from two consecutive generations (2 x 47 individuals) were tested for SRE using the qPCR assay described in Materials and Methods.

Plutella xylostella individuals were supplied from a rearing at NIBIO. The rearing conditions were 18 °C and 70 % relative humidity at an 18/6 h day/night cycle. Adults lay eggs after approx. 1 week, the eggs hatch after 4-7 days and pupate after approx. 14 days. Pupae hatch after 9-12 days. The number of adults kept in one cage was kept stable at approximately 80 individuals. Adult *P. xylostella* were fed with honey water (changed 2x / week) and were supplemented with brassica plants (mostly Chinese cabbage, *Brassica rapa* subsp. *pekinensis*) grown in clean plant rooms for egg deposition. Eggs, including plant material were transferred to new cages. The remaining leaves were removed and discarded when a sufficient amount of pupae was present to sustain the rearing. For the assessment of the presence of SRE, 94 individuals from one generation were tested.

Chrysoperla carneum larvae were obtained as commercial products from five different retailers and eight larvae were tested from each producer. The products and their producers were Chrysopha (Koppert), Biocarn (BioProduction), Chrysopa-System (Biobest), Chrysoline C (Syngenta Bioline) and MC-500 (Borregaard BioPlant).

References

1. Woodward EJ, Robinson K (1990) An improved formulation and method of preparation of crystal violet pectate medium for detection of pectolytic *Erwinia*. *Lett Appl Microbiol* 10(4):171-173.