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

2   potato fields

3   Simeon Rossmann,[a,b] Merete Wiken Dees,[a] Juliana Perminow,[a] Richard Meadow,[a,b] May

4   Bente Brurberg [a,b]#

5   [a]Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research

6   (NIBIO), Ås, Norway.

7   [b]Department of Plant Sciences, Norwegian University of Life Sciences (NMBU), Ås, Norway

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

10   #Address correspondence to May Bente Brurberg, may.brurberg@nibio.no

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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 initially pathogen-free tubers 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 two years. Twenty-eight yellow sticky traps were set up in ten potato fields throughout Norway to attract and trap insects. Total DNA recovered from over 2000 randomly chosen trapped insects was tested for SRE, using a specific 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 highest amount of SRE, more than 80 other SRE-carrying insect species were identified, in varying numbers. 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, compared to cabbage moth (*Plutella xylostella*) and carnivorous green lacewing larvae (*Chrysoperla carnea*). In summary, the high proportion, variety and ubiquity of insects that carried SRE shows the need to address this source of the pathogens to reduce 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

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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.

Introduction

44

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).

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

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

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.

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).

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.

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).

176

177

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).

11

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

12

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),

13

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

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.

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.

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

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

18

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*.

19

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

391 **Acknowledgements:**

397

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510

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

24

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.**

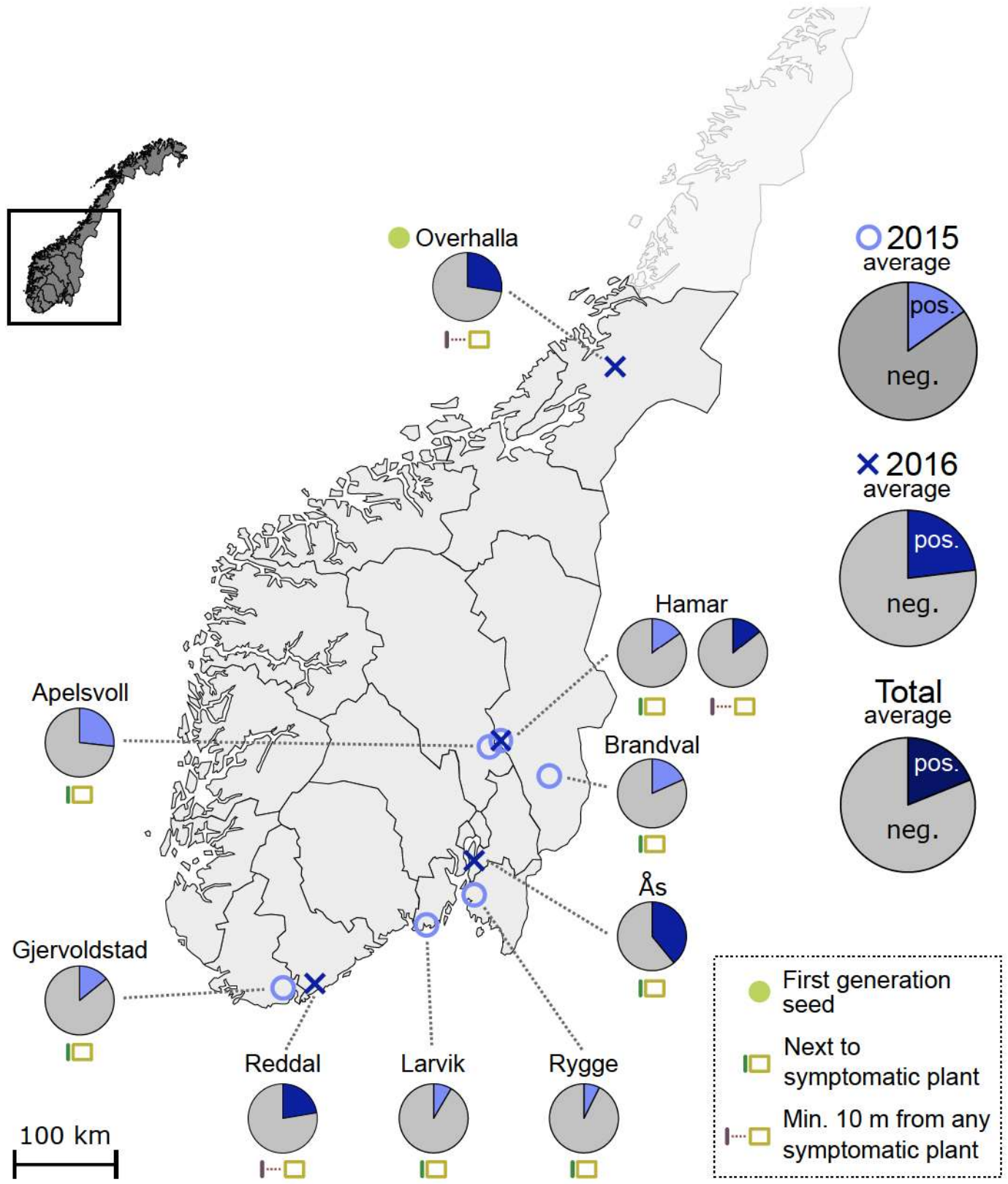| Target	Name	Forward sequence (5'-3')	Reverse sequence (5'-3')	TaqMan probe (5'-3')	Reference
All SRE	PEC	GTGCAAGCGTTAATCGGAATG	CTCTACAAGACTCTAGCCTGTCAGTTTT	CTGGGCGTAAAGCGCACGCA	(23)
Dickeya solani	SOL-C	GCCTACACCATCAGGGCTAT	ACACTACAGCGCGCATAAAC	CCAGGCCGTGCTCGAAATCC	(23)
Pectobacterium atrosepticum	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)

25

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)
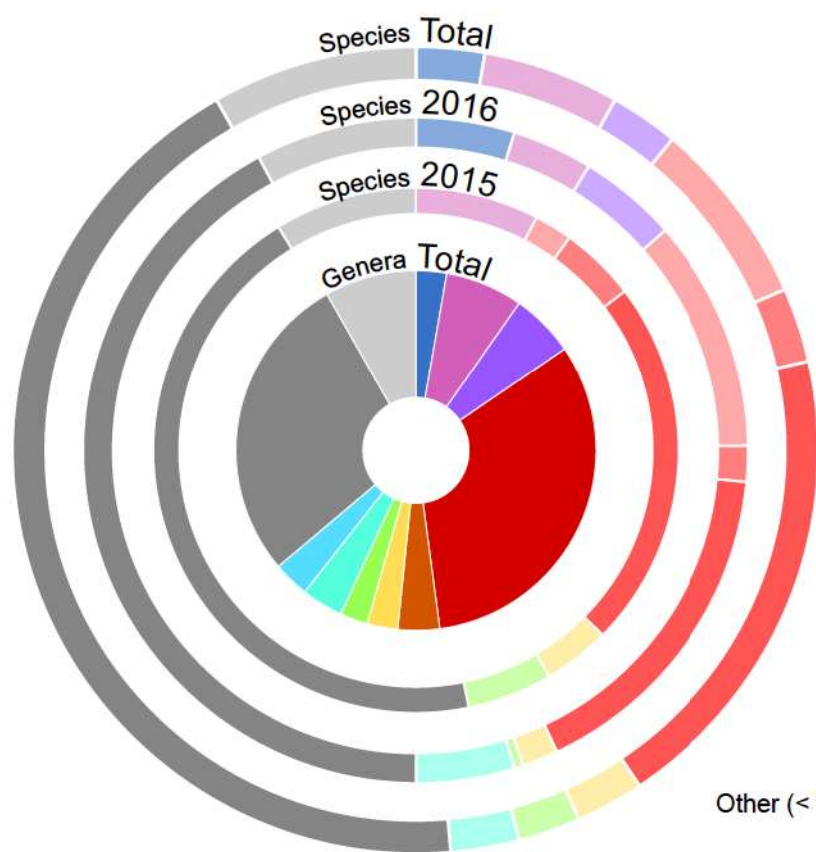
535   highlighted in black, and others in light grey.

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

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.

Overhalla

Apelsvoll

Gjervoldstad

100 km

Reddal

Larvik

Rygge

Hamar

Brandval

Ås

○ 2015
average
pos.
neg.

✕ 2016
average
pos.
neg.

Total
average
pos.
neg.

● First generation seed

Next to symptomatic plant

Min. 10 m from any symptomatic plant

A

B