Supplemental material

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

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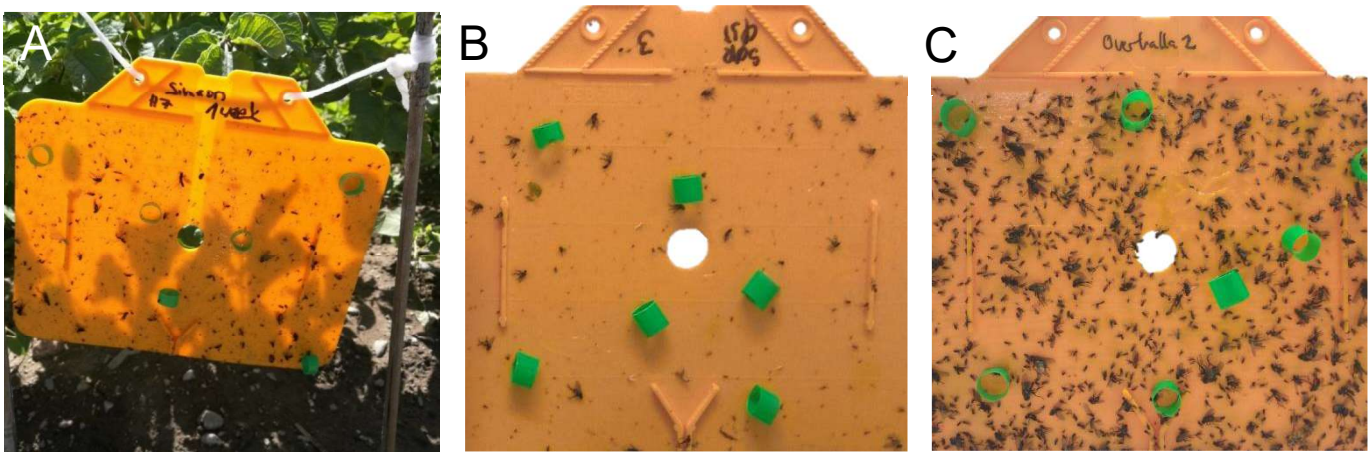

Fig. S1. Yellow sticky traps before processing. All traps are shown after having been in various fields for approximately one week. (A) Trap on field in Ås. (B) Trap number three from Rygge, collected in 2015 (Rygge 3) with relatively few insects on it. (C) Trap number two from the Overhalla field for 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.

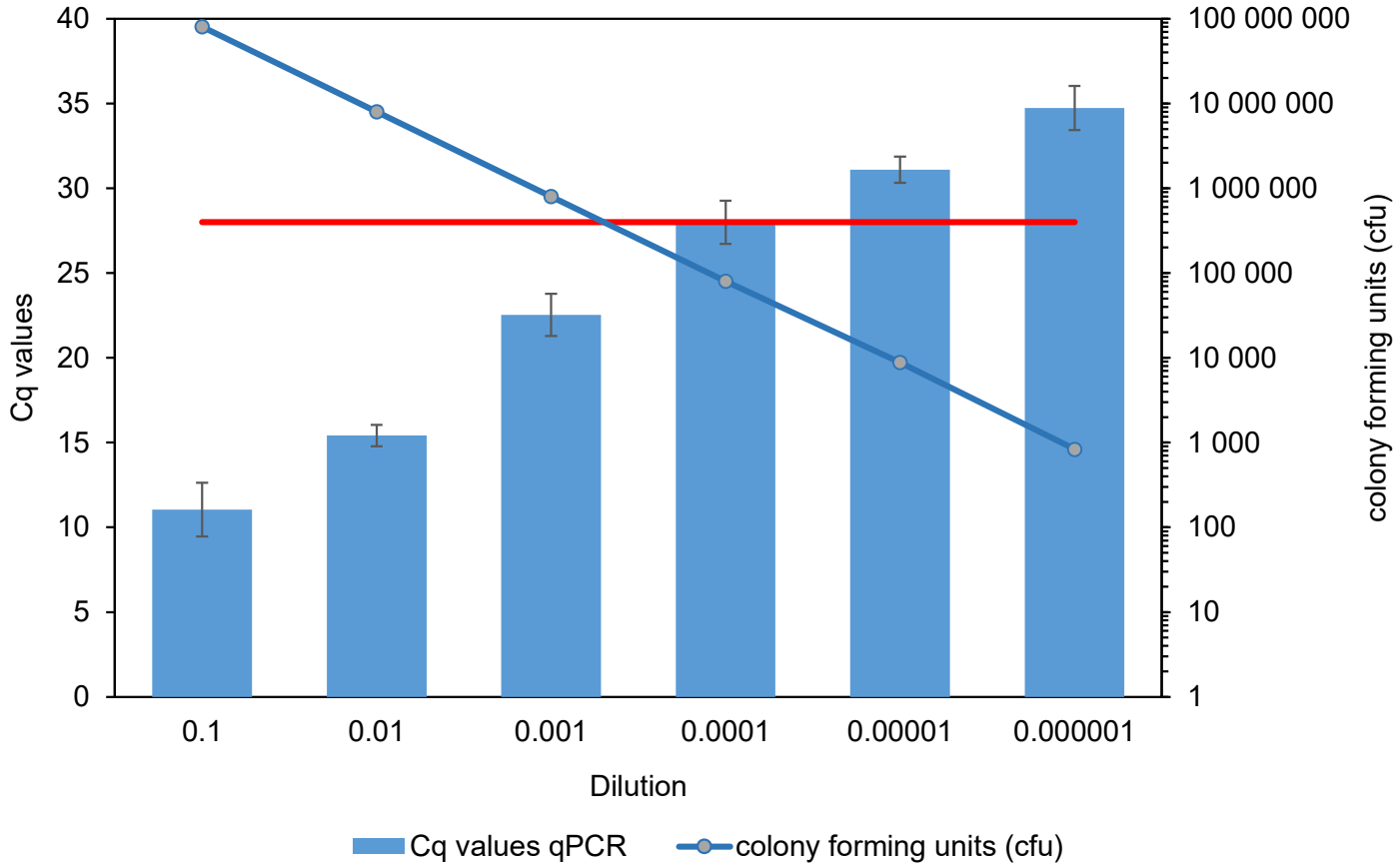

Fig. S2. Relationship between the Cq values obtained in the PEC qPCR assay and number of cfu after plating in a dilution series of *P. polaris* (strain NIBIO1006) on LB agar-plates. The bars show the average Cq values obtained in the PEC qPCR assays from three dilution series, each tested in three PCR 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.