SUPPLEMENTARY INFORMATION

First detection of a *Sesamia nonagrioides* resistance allele to Bt maize in Europe

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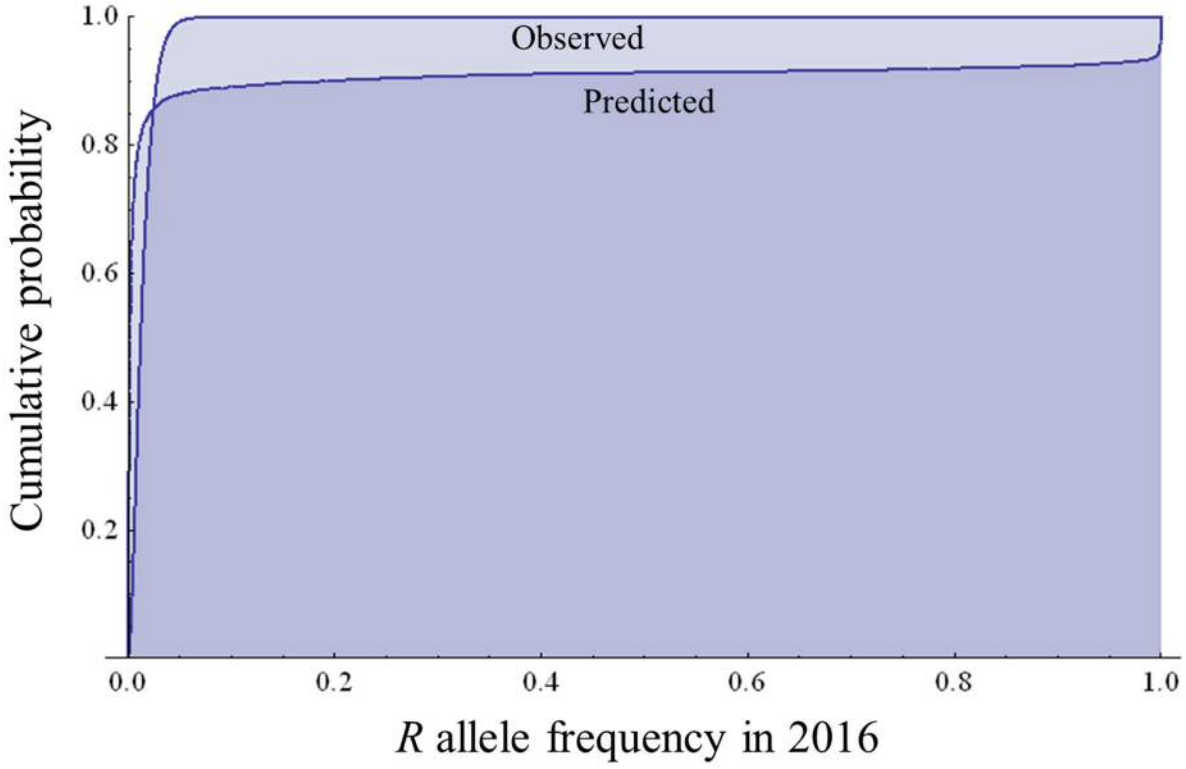
Supplementary Table S1. Type II error (P_{No}) was calculated for each line that was subjected to the F_2 screen based on the number of F_1 females and males and the number of F_2 neonates screened on Bt maize per female. Only 385 lines were part of the experiment even though 418 pairs of adults were initially in the parental generation, as 33 lines were randomly discarded during the first stage of the F_1 generation due to logistical issues.

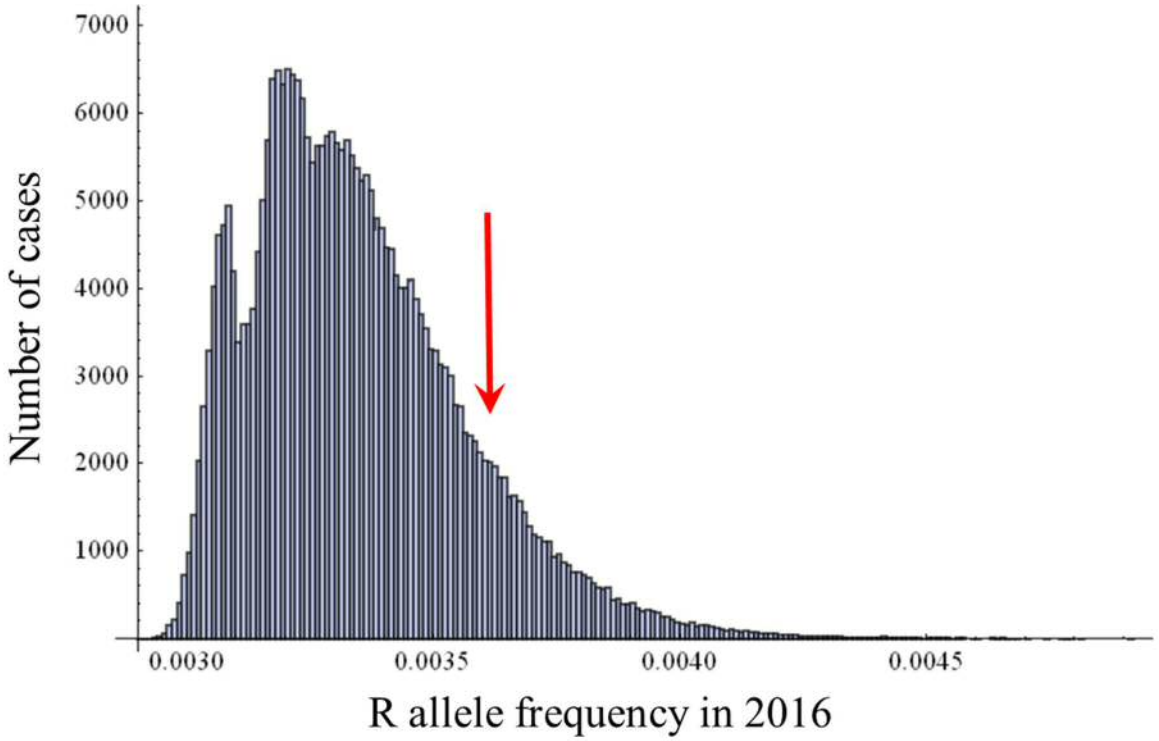
Line number	F_1 females (F)	F_1 males (M)	Number of neonates screened per F_1 female (J)	Control mortality (%)
6	23	34	10.9	12
9	31	43	8.1	4
12	11	7	22.7	24
13	26	22	9.6	0
18	20	34	12.5	0
20	8	10	31.3	10
22	33	33	7.6	22
27	34	28	7.4	18
28	26	36	9.6	10
29	27	24	9.3	10
31	24	26	10.4	14
33	28	24	8.9	26
34	17	14	14.7	26
38	27	32	9.3	28
40	16	24	15.6	12
44	35	26	7.1	8
45	7	9	14.3	20
46	21	20	11.9	2
49	24	10	10.4	0
50	14	28	17.9	14
51	5	9	50.0	10
55	26	25	9.6	32
59	27	36	9.3	4
67	26	13	9.6	16
68	7	5	24.3	32
74	35	25	7.1	14
78	27	20	9.3	30
79	15	15	16.7	38
93	11	26	22.7	0
100	36	55	6.9	18
104	41	38	6.1	10
106	28	39	8.9	8
108	35	42	7.1	18
109	36	46	6.9	42
112	15	7	16.7	4
113	17	21	14.7	6
115	18	21	13.9	8

123	32	39	7.8	10
124	32	24	7.8	20
126	40	26	6.3	16
129	38	25	6.6	12
132	26	30	9.6	12
133	8	7	31.3	8
134	54	47	4.6	6
137	29	27	8.6	12
138	29	25	8.6	6
139	12	10	20.8	24
141	22	12	11.4	4
145	37	37	6.8	22
150	26	35	9.6	26
153	39	50	6.4	8
156	36	52	6.9	16
162	23	25	10.9	10
164	40	34	6.3	10
165	36	29	6.9	18
167	17	12	14.7	60
168	37	34	6.8	6
171	26	29	9.6	26
172	29	31	8.6	10
173	60	55	4.2	0
176	36	25	6.9	28
189	10	15	25.0	2
190	22	20	11.4	6
191	22	35	11.4	16
192	40	22	6.3	24
194	22	30	11.4	12
196	26	35	9.6	8
201	23	20	10.9	2
204	24	24	10.4	12
205	31	26	8.1	14
210	23	31	10.9	0
211	7	3	35.7	4
212	7	7	8.6	12
216	40	33	6.3	8
218	37	41	6.8	10
220	35	26	7.1	4
226	15	22	16.7	36
229	12	11	20.8	20
232	54	28	4.6	22
236	31	32	8.1	16
237	43	53	5.8	6
247	44	28	5.7	8
255	35	25	7.1	10
256	39	37	6.4	12
260	38	43	6.6	8
261	9	17	27.8	6
263	10	3	16.5	4
266	19	23	13.2	2
268	22	15	11.4	10

269	38	22	6.6	18
272	18	28	13.9	14
274	21	27	11.9	4
276	35	34	7.1	14
277	29	31	8.6	10
278	28	28	8.9	0
289	17	12	14.7	18
296	45	37	5.6	28
297	36	43	6.9	14
301	54	52	4.6	20
302	17	16	14.7	14
303	18	24	13.9	10
305	10	9	25.0	0
308	29	36	8.6	4
309	38	33	6.6	24
312	22	11	11.4	4
313	12	18	20.8	6
314	47	26	5.3	12
316	27	20	9.3	6
323	26	22	7.6	14
328	57	27	4.4	16
329	33	29	7.6	24
330	23	28	10.9	8
331	42	28	5.6	22
338	49	63	5.1	16
341	12	15	20.8	6
345	14	18	17.9	6
347	11	6	22.7	14
350	37	48	6.8	18
352	22	37	11.4	16
360	17	20	14.7	16
362	19	35	13.2	16
363	44	38	5.7	0
375	32	42	7.8	4
378	32	25	7.8	14
380	34	30	7.4	6
381	25	30	10.0	22
385	4	4	15.0	15
392	45	33	5.6	0
394	9	13	27.8	12
396	63	40	4.0	8
400	26	38	9.6	30
402	23	21	10.9	16
404	24	46	10.4	4
406	42	46	6.0	32
407	40	32	6.3	14
409	26	34	9.6	2
412	26	26	9.6	6
Mean	27.4	27.2	11.4	13.2
s.e.m.	1.0	1.0	0.6	0.8



Supplementary Figure S1. Predicted and observed cumulative probability distributions for the R allele frequency in 2016. The observed distribution was based on the posterior beta distribution estimated in this paper. The predicted distribution was estimated from 10,000 Monte Carlo simulations of the *Sesamia nonagrioides* resistance evolution model[1], with initial R allele frequencies randomly selected from the beta distribution in Andreadis *et al*. (2007)[2] and updated with the area of Bt maize for 2014-2016. The two distributions are different, and the prediction is poor. The predicted distribution has about 5% of all cases with the R allele going to fixation by 2016 and about 10% of the cases the frequency is >0.5. Clearly, this has not happened. It also over-predicts the probability of a low R allele frequency in 2016. These results suggest that the initial R allele frequency distribution was poorly estimated, probably because only 160 F_2 lines were screened. This resulted in an initial probability distribution for the R allele frequency with a long tail, with significant probability of an R allele frequency >0.004, which was predicted to lead to rapid resistance failure.



Supplementary Figure S2. Histogram of the predicted probability distribution for the *R* allele frequency in 2016. The predicted distribution was estimated from 300,000 Monte Carlo simulations of the *Sesamia nonagrioides* resistance evolution model[1], with the fixed initial *R* allele frequency = 0.0015 and updated with the area of Bt maize for 2014-2016. The mean of this distribution is 0.003347. Red arrow is the estimated *R* allele frequency estimated in this work (0.003617). The probability that the estimated frequency is from the predicted distribution is 0.1157 (ns). This suggests that the original estimated initial *R*-allele frequency may have been accurate. Although not statistically different, the current estimated frequency is higher than the predicted frequency for 2016.

Supplementary Text. As a geographic unit, the Ebro Valley includes parts or all of different Autonomous Communities in northeast Spain, the largest of which are Aragón and Cataluña. These two also are the major producers of maize in the Ebro Valley, where adoption of Bt maize reached 76.9% during 2016. The evolution of resistance in *Sesamia nonagrioides* was modeled in these two regions (Castañera *et al*. 2016). Recently, adoption of Bt maize has increased dramatically in Navarra, an Autonomous Community upstream and to the west of Aragón. The proportion of Bt maize in Navarra nearly tripled in the last decade, from 21% in 2004-2005 to 54% in 2016. Consequently, in this study, we have included Navarra as a part of the Ebro Valley. Navarra contributes ~15% (2015) of the maize area in the Ebro Valley.

References for Supplementary Information

1. Castañera, P., Farinós, G. P., Ortego, F. & Andow, D. A. Sixteen years of Bt maize in the EU hotspot: Why has resistance not evolved? PLoS ONE. **11**(5), e0154200.doi:10.1371/journal. pone.0154200 (2016).
2. Andreadis, S. S. *et al*. Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in Greek and Spanish population of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *J. Econ. Entomol*. **100**(1)**,** 195-201 (2007).