Supplemental Information: Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*

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Supplemental Experimental Procedures

Samples of wax worms. Two sources of wax worms of the moth *G. mellonella* were used: environmentally bred worms from the Spanish countryside (Cantabria), and commercially bred worms from Hobby Zoo Pinto shop (Spain).

Sample of PE. PE was sourced from commercially available PE plastic bags (Marks and Spencer, 2015).

Preparation of the wax worm homogenate. The crude wax worm extract was made by homogenising fresh worms in a mortar at low temperature (0-4 °C). The resulting paste was then smeared on the surface of a film of PE and left in contact for a certain amount of time as detailed in the appropriate experimental section. The thickness of the smeared paste was about 0.5 cm.

Biodegradation of a commercial PE shopping bag. The results shown in figure 1A and 1B were obtained as follows. ~100 wax worms were left in contact with a commercial PE shopping bag. The bag was weighed initially (2730 mg); after incubation worms were picked off the bag, the bag was cleaned with deionized water, carefully dried, and then finally re-weighed (2638 mg).

Gravimetric analysis of treated PE samples. The results shown in figure 1C were obtained as follows. The crude wax worm homogenate was made as described above. The resulting paste was smeared on the surface of several films of PE and left in contact for 2 hours at room temperature. Then, the paste was gently removed and replaced with a fresh layer of wax worm homogenate. The routine was repeated 7 times for a total of 14 hours. The samples were cleaned with deionized water and carefully dried, and finally weighed. Untreated sample of PE underwent the same protocol of washing and drying. The mass per unit area was determined before and after treatment.

FTIR analysis. The results shown in figure 1D and 1E were obtained as follows. The crude wax worm homogenate was made as described above. The homogenate was smeared on the surface of several films of PE and left in contact for 2 hours at room temperature. The samples were cleaned with deionized water and carefully dried.

Untreated sample of PE underwent the same protocol of washing and drying. Films that had been treated with homogenate and un-treated controls were analysed by ATR FTIR to characterise the results of breakdown. A iS50 ATR apparatus (Thermo Scientific, USA) was used. The samples were placed face down on the ATR crystal and scanned between 700 to 4000 cm^{-1}. For each sample, the background was corrected and four spectra were taken and averaged.

HPLC-MS analysis. The results shown in Supplementary figure 1G were obtained as follows. The crude wax worm homogenate was made as described above. The homogenate was smeared on the surface of several films of PE and left in contact for 24 hours at room temperature. The samples were carefully cleaned with deionized water and dried. Untreated sample of PE underwent the same protocol of washing and drying. Both the treated and untreated PE samples were analysed by HPLC-MS (Waters ZQ mass spectrometer with a Waters 2795 HPLC). The samples were submerged in acetonitrile and sonicated for around 1 minute. Then, the PE was removed and the solvent evaporated using a vacuum. The soluble products were then dissolved in 1 ml of fresh acetonitrile, which was then transferred to a microcentrifuge tube and spun down for 2 minutes. The supernatants of the untreated and treated samples were then placed in HPLC vials and run via LCMS. The chromatograms shown in the Supplementary Figure 1F display the total ion current (TIC) *versus* the elution time for the solvent alone (acetonitrile) untreated and treated samples respectively. An increase in these indicates an increase in current at the mass spectrometer detector as will be observed when a compound elutes from the column. The difference between the traces, untreated and treated is the peak observed at 5.75 minutes. This peak is only observed in the treated sample. The untreated sample has a TIC that is essentially identical to the solvent alone (acetonitrile). The mass spectra reported in the figure 1H are derived from the fractions eluted at 5.75 minutes for the untreated and treated samples.

Atomic Force Analysis (AFM). The results shown in figure 1F and 1G were obtained as follows. The crude wax worm homogenate was made as described above. The homogenate was smeared on the surface of several films of PE and left in contact for 2 hours at room temperature. The samples were cleaned with deionized water and carefully dried. Untreated sample of PE were subjected to the same protocol of washing and drying. Both the treated and untreated samples were analysed by a commercial AFM system (Anasys Instruments, USA). Samples were scanned with a line rate between 0.1-0.3 Hz in contact mode with a silicon cantilever (AppNano) having a

nominal radius of 10 nm and spring constant of 0.5 N/m. Images were acquired with at least a resolution of 500 × 500 pixels per image. The AFM images were processed using Scanning Probe Image Processor (SPIP)-6.3.4. The morphology maps were first flattened, then their roughness was evaluated by SPIP. The roughness of the different areas, for a total of 75 um^2, was averaged to compare the control and treated samples. All measurements were performed at room temperature.

Statistical validation. One-way analysis of variance (ANOVA) was used to determine whether there were any significant differences between the means of independent (unrelated) groups of data. When the p-value was greater than 0.05 there was no statistically significant difference between group means**.** The complete results obtained from the ANOVA tests run in this study are shown in the Supplementary Table 1. The results were calculated by using online software available at:

http://www.danielsoper.com/statcalc3/calc.aspx?id=43

(Accessed: 6th February 2016).

Given the mean, standard deviation, and (n) in each group, p value is calculated by an ANOVA.

SS: sums of squares;

df: degrees of freedom;

MS: mean squares;

F and p-values.

Author Contributions

P. B., F. B. and C. J. H. designed the experiments, P.B. and F.B. conducted the experiments, P.B., F.B., and C.J.H. wrote the paper.

Supplemental Figure 1

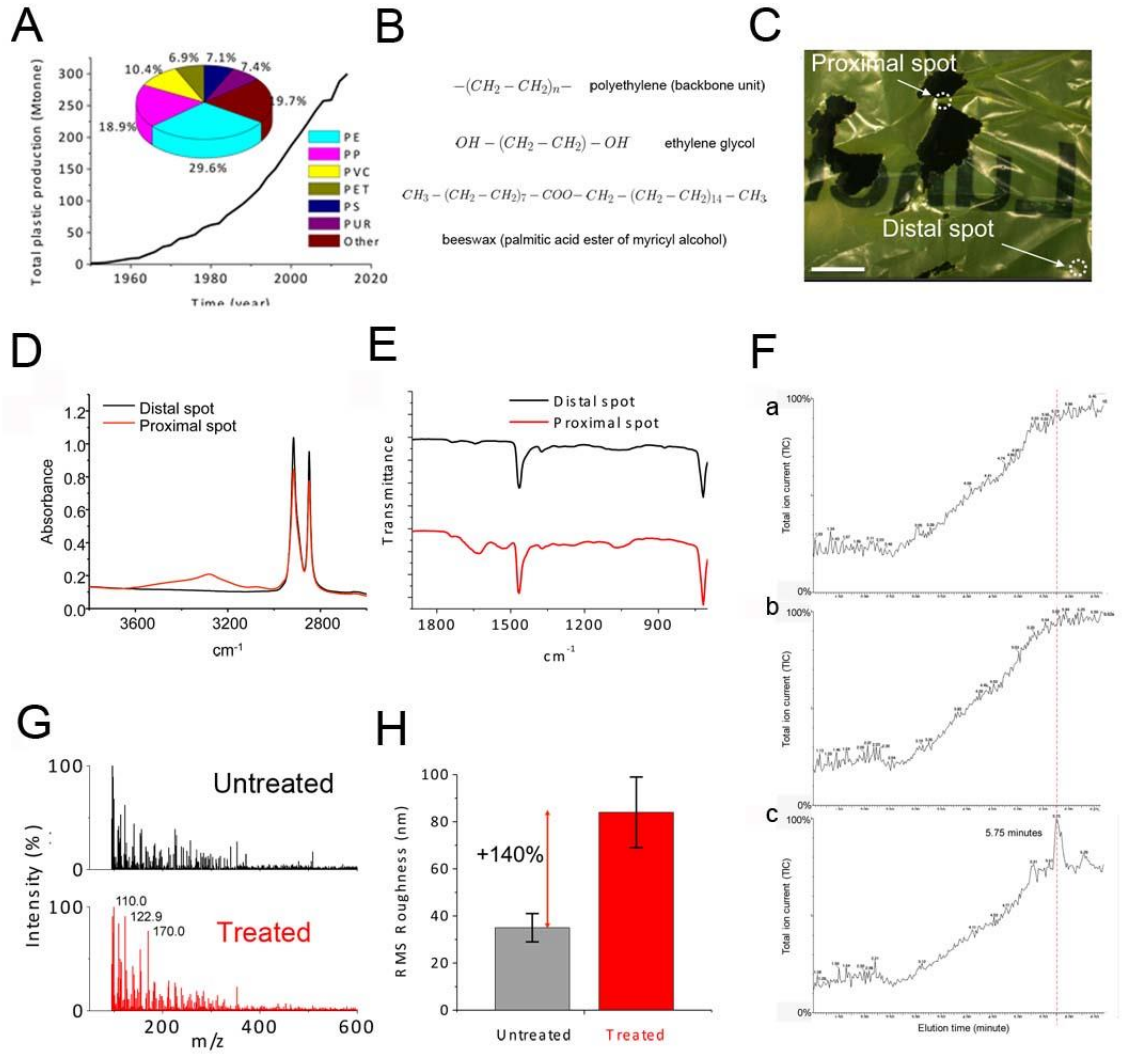

Figure S1. A. The black line represents the increase (in millions of tons) in plastic production worldwide in the past 50 years (http://discardstudies.com, accessed: 4th February 2016). Inset: Pie chart shows the diffusion of plastics classified by polymer type (PE, polyethylene; PP, Polypropylene; PVC, Polyvinyl Chloride; PET, Polyethylene Terephthalate; PS, Polystyrene; PUR, polyurethane). **B.** Chemical formulae of polyethylene (PE), ethylene glycol and palmitic acid ester of myricyl alcohol, one of the multiple compounds that constitute beeswax. **C.** PE degraded film (holes) after exposure to the wax worm. Scale bar: 5mm. **D** and **E.** FTIR analysis of the PE film. **F.** Chromatograms for the total ion current (TIC) *versus* the elution time for the solvent alone (acetonitrile) (a) untreated (b) and treated

(c) samples. **G.** Mass spectroscopy analysis of homogenate-treated and control PE. In the sample treated with the wax worm extract three new peaks at lighter m/z appear (110.0, 122.9 and 170.0). **H.** AFM of homogenate-treated and control PE. The histogram represents distinct measurements (n=3 mean ± standard error) of treated (red column) and untreated (grey column) PE film. Treated PE showed an increase of roughness calculated as % of treated sample.

Supplemental Table 1

a)

# worms	# holes	time(h)	hole worm^{-1} h^{-1}
1	6	4	1.50
1	15	24	0.63
1	3	3	1.00
1	3	3	1.00
1	3	3	1.00
1	10	4	2.50
1	20	4	5.00
1	10	4	2.50
1	2	2	1.00
1	9	5	1.80
1	16	5	3.20
1	9	3	3.00
1	13	9	1.44
1	15	5	3.00
6	140	48	2.92
		Average	**2.10**
		St.dv.	***1.20***

Quantitative estimation of hole formation when a PE film was left in direct contact with worms. Individual (or in one case, several) worms were left in contact with the film, and the number of holes counted at different time points. Each line in the table represents a separate experiment.

b)

	(before)	(after)		Before t.	After t.	Delta
	mg	mg	cm^2	mg cm^{-2}	mg cm^{-2}	mg cm^{-2}
1	3.93	3.36	4	0.982	0.839	0.143
2	3.70	3.55	4	0.926	0.887	0.039
3	4.52	3.62	4	1.129	0.905	0.224
4	4.66	3.80	4	1.164	0.950	0.214
5	4.44	3.79	4	1.110	0.948	0.162
6	3.58	3.41	3	1.193	1.136	0.058
7	3.49	3.17	3	1.164	1.058	0.107
			Average	**1.096**	**0.960**	**0.135**
			St.er.	***0.038***	***0.039***	***0.027***
			St.dv.	***0.102***	***0.103***	***0.072***

Gravimetric analysis carried out as described in material and methods.

c)

	SS	Df	MS	F	p
Between:	0.065	1	0.065	6.162	**0.029**
Within:	0.126	12	0.011		
Total:	0.191	13			

Statistical validation of the gravimetric analysis carried out as described in material and methods.

d)

	SS	Df	MS	F	p
Between:	3,645.7	1	3,645.7	30.07	**0.005**
Within:	485.03	4	121.26		
Total:	4,130.8	5			

Atomic Force Microscopy was carried out on 3 separate independent experiments as described in material and methods.