**Table S1.** Identification codes and general information for the collected *L. botrana* populations

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Country** | **Population** | **Location** | **Coordinates** | **Sampling data** |
|
| Reference |  LB-S |  |  | June, 2015 |
| Greece | BAG 21-1 | Bagiona, Crete |  35° 1'28.66"N, 24°58'53.56"E | June, 2021 |
| EPIS 21-4 | Episkopi, Crete |  35°15'23.23"N, 25°14'25.02"E | July, 2021 |
|  | ARC 19-2 | Archanes, Crete | 35°14'35.74"N,25°9'48.07"E | June, 2019 |
|  | EPIS 19-3 | Episkopi, Crete | 35°15'46.43"N,25°13'31.26"E | July, 2019 |
|  | ALA 19-6 | Alagni, Crete | 35°11'1.30"N,25°12'42.36"E | Sep., 2019 |
|  | ARC 20-1 | Archanes, Crete | 35°14'47.5"N,25°09'44.4"E | July, 2020 |
|  | AAR 20-2 | Ano Archanes, Crete | 35°13'35.9"N,25°09'37.7"E | July, 2020 |
| Turkey | R | Manisa-Merkez |  38°40'27.35"N, 27°24'29.44"E | July, 2017 |
| MA | Manisa-Alaşehir |  38°21'23.97"N, 28°31'21.25"E | July, 2017 |
| MAh | Manisa-Ahmetli |  38°30'57.73"N, 27°56'17.71"E | July, 2018 |
| MS | Manisa-Saruhanlı |  38°44'15.06"N, 27°34'28.55"E | July, 2018 |
| D | Denizli |  37°52'10.18"N, 29° 1'51.42"E | July, 2017 |
| S | Kahramanmaraş |  37°33'34.06"N, 36°33'27.37"E | July, 2017 |

**Table S2.** Validation of the Modified IRAC017 bioassay protocol by comparing the probit analysis output to the standard IRAC017 protocol for the insecticides tested. The Log dose probit mortality data analysis on the susceptible reference strain (LB-S) are presented.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Insecticide** | **Bioassay method** | **N** | **LC50** | **CL 95%** | **Slope** | **s.e.** | **X2** | **df** | **p** |
| Chlorantraniliprole | Modified IRAC017 | 93 | 0.09 | 0.05-0.14 | 2.53 | 0.73 | 1.91 | 2 | 0.38 |
| Standard IRAC017 | 114 | 0.08 | 0.03-0.17 | 2.72 | 1.24 | 4.70 | 2 | 0.10 |
| alpha- cypermethrin | Modified IRAC017 | 99 | 0.35 | 0.22-0.39 | 3.14 | 0.72 | 2.71 | 2 | 0.26 |
| Standard IRAC017 | 118 | 0.36 | 0.3-0.45 | 4.23 | 0.46 | 2.15 | 3 | 0.54 |
| Spinetoram | Modified IRAC017 | 108 | 0.06 | 0.04-0.07 | 5.68 | 1.48 | 0.09 | 2 | 0.96 |
| Standard IRAC017 | 110 | 0.04 | 0.01-0.09 | 3.65 | 1.29 | 5.82 | 2 | 0.05 |
| Spinosad | Modified IRAC017 | 112 | 0.23 | 0.19-0.27 | 5.30 | 1.22 | 5.70 | 2 | 0.06 |
| Standard IRAC017 | 112 | 0.37 | 0.27-0.49 | 5.36 | 0.65 | 1.65 | 2 | 0.44 |
| Etofenprox | Modified IRAC017 | 89 | 77.7 | 40.9-96.8 | 5.70 | 1.91 | 0.23 | 1 | 0.63 |
| Standard IRAC017 | 185 | 32.5 | 25.3-43.2 | 2.63 | 0.69 | 4.04 | 3 | 0.26 |
| Acetamiprid | Modified IRAC017 | 119 | 24.1 | 19.1-27.9 | 9.34 | 3.17 | 1.47 | 2 | 0.48 |
| Standard IRAC017 | 117 | 15.6 | 10.2-29.3 | 1.73 | 0.37 | 1.86 | 3 | 0.60 |

**Table S3.** The target genes of different insecticides that were examined in the Greek and Turkish *L. botrana* populations for potential point mutations, in known positions, associated in other insects with insecticide resistance.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Insecticide groups** | **Target genes** | **Resistance mutations** | **Primer Name** | **Sequence (5’–3’)** | **Amplified****product (bp)** | **Annealing T (°C)** | **Literature** |
| Diamides | Ryanodine receptor (RyR) | I4746M/T, G4903V/E | Lbot\_RyR\_F1 | TAGACGAGGATTACTTCTACATGGA | 690 | 58 | Roditakis et al., 2017a |
| Lbot\_RyR\_R1 | CCTCCTCCTGCACGTAGAACTTG |
| Pyrethroids | Voltage gated sodium channel (VGSC) | M918T/L/V/I, L925I/V, T929I/C/V/N, L932F, G933V, I936V, G943A, Q945R, F979S, I1011M/V, N1013S, L1014F/S/H/C/W, V10106G/I, F1020S | Lbot\_vgsc\_\_kdr\_F1 | GCTTCGAGTATTCAAATTGGCA | 394 | 57 | Reviewed by Dong et al., 2014 |
| Lbot\_vgsc\_\_kdr\_R1 | GCCAAAATTTGACAGTAACAGGG |
| Indoxacarb | F1845Y, V1848I | Lbot\_vgsc\_\_indo\_F2 | ACAACTTCAAGACTTTCGTGCAGAG | 357 | 60 | Roditakis et al., 2017b |
| Lbot\_vgsc\_\_indo\_R3 | GCTGGTCGTAGCGGATGTACTG |
| Organophosphates | Acetylcholinesterase (ace) | G119S, D128E, V150L, A201S, G227A, F290V, G328A, F331W/C/Y/H, L336S | Lbot\_ace\_F1 | CACACCTAGACCCAGGCCAA | 806 | 55 | Reviewed by Feyereisen et al., 2015 |
| Lbot\_ace\_R1 | CTTGTCGGGCCTCATCAGTC |
| Spinosyns | Nicotinic acetylcholine receptor a6 subunit (nAChR\_α6) | G275E | Lbot\_a6\_F4 | CCTCAACCTGGTAGCCGAGAC | 174 | 60 | Silva et al., 2016 |
| Lbot\_a6\_R3 | GCAGTGCGGTGGTGGTAGTT |
| Avermectins | Glutamate-gated chloride channel (GluCl) | G314D/G326E, I321T, A309V | Lbot\_GluCl\_F1 | TTCCACAACATCATCATGCC | 824 | 57 | Dermauw et al., 2012; Xue et al.,2020; Wang et al., 2016 |
| Lbot\_GluCl\_F1 | GGGAACTTGGACATCCAGAG |

**Table S4.** Transcriptome assembly summary

|  |  |
| --- | --- |
| **Number of transcripts** | **169,945** |
| **Number of unigenes** | **98,064** |
| **Predicted peptides** | **43,857** |
| **with a BLAST hit vs Uniref50, e-value <10-5** | **39,341** |
|  **against Metazoa** | **34,285** |
|  **against Arthropoda** | **32,880** |
|  **against Lepidoptera** | **26,364** |
|  **against Bacteria** | **2,109** |
| **Filtered gene set** | **22,803** |
|  **nearly full-length genes (from Uniref50)** | **3,279** |
|  **with a Pfam domain (from InterProScan)** | **13,870** |
| **BUSCO quality assessment (transcriptome)** | - |
|  **Number of complete Insecta BUSCOs** | **1,393 (84.0%)** |
|  **Number of fragmented Insecta BUSCOs** | **197 (11.9%)** |
|  **Number of missing Insecta BUSCOs** | **68 (4.1%)** |

**Table S5.** Number of *L. botrana* and *H. armigera* CYPs per clan

|  |  |  |
| --- | --- | --- |
| **Clan** | ***L. botrana*** | ***H. armigera*** |
|  | **Total** | **Full-length** |
| **MITO** | 16 | 4 | 10 |
| **Clan 2** | 1**1** | 4 | 8 |
| **Clan 3** | 8**5** | **30** | 45 |
| **Clan 4** | 49 | 11 | 49 |
| **Total** | **161** | **48** | **112** |