Accession Number		Strain	Locality (Governorate)	Coordinates		Sensitiv	vity	Amino acid
nucleotide	protein	designation				R/S		392 ∏
LC199300.1	BAW19578.1	Egy-B (res. lab)				R		W
LC199301.1	BAW19579.1	HS-S (sus. lab)		Х	Υ	9	5	F
LC199302.1	BAW19580.1	Beb	Beheira	3418867.71	280123.626	R		W
LC199303.1	BAW19581.1	Bwn	Beheira	3362316.078	246182.98	9	5	F
LC199304.1	BAW19582.1	Dmg	Dakahlia	3394642.967	335258.342	9	5	F
LC199305.1	BAW19583.1	Dbq	Dakahlia	3454816.031	341340.995	R		W
LC199306.1	BAW19584.1	Fsn	Faiyum	3257451.651	295005.12	9	5	F
LC199307.1	BAW19585.1	Fas	Faiyum	3234668.212	285418.568	R		W
LC199308.1	BAW19586.1	Fib	Faiyum	3248639.659	274272.467	R		W
LC199309.1	BAW19587.1	Gzf	Gharbia	3396906.492	330704.557	9	5	F
LC199310.1	BAW19588.1	Gkt	Gharbia	3423020.657	304956.928	R		W
LC199311.1	BAW19589.1	Qqb	Qalyubia	3339945.873	326113.753	9	5	F
LC199312.1	BAW19590.1	Qbn	Qalyubia	3376932.623	328268.213	R		W
LC199313.1	BAW19591.1	Mym	Minya	3069341.048	283897.883	R		W
LC199314.1	BAW19592.1	Myi	Minya	3175623.75	283504.66	R		W
LC199315.1	BAW19593.1	Mys	Minya	3123464.44	274247.874	9	5	F
LC199316.1	BAW19594.1	Mbs	Menoufia	3390934.3	320834.183	R		W
LC199317.1	BAW19595.1	Mas	Menoufia	3350187.134	306771.746	R		W

Supplementary Information S1. GenBank accession numbers of the silverleaf whitefly *Bemisia tabaci ace-1* genes. Field strain designation, the predicted sensitivity^{*} (R: resistant; S: susceptible) of the analyzed *B. tabaci* populations from Egypt are provided

*Based on Alon et al. (2008): designation of *B. tabaci ace1*-type acetylcholinesterase sensitivities to different insecticides. Arrangement order of localities follows the descending order of the assigned GenBank accessions.

^{II} As per Alon et al. (2008), the substitution of the amino acid residue phenylalanine (F) with tryptophan (W) (F392W) confers resistance to organophosphates, *i.e.*, the resistant alleles have tryptophan in this position of the full-length AChE1 protein sequence, while susceptible alleles retain the phenylalanine residue. The same nomenclature was used by Zhang et al. (2012) and Hopkinson et al. (2020).

Alon M, Alon F, Nauen R, Morin S (2008) Organophosphates' resistance in the B-biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) is associated with a point mutation in an *ace1*-type acetylcholinesterase and overexpression of carboxylesterase. Insect Biochem Mol Biol 38:940–949. <u>https://doi.org/10.1016/j.ibmb.2008.07.007</u>

Hopkinson J, Pumpa S, van Brunschot S, Fang C, Frese M, Tay WT, Walsh T (2020) Insecticide resistance status of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production valleys. Austral Entomol 59:202–214. <u>https://doi.org/10.1111/aen.12436</u>

Zhang N, Liu C, Yang F, Dong S, Han Z (2012) Resistance mechanisms to chlorpyrifos and F392W mutation frequencies in the acetylcholine esterase ace1 allele of field populations of the tobacco whitefly, *Bemisia tabaci* in China. J Insect Sci 12:41. <u>https://doi.org/10.1673/031.012.4101</u>



Supplementary Information S2. Alignment of the AChEs' prototype model (*Torpedo californica* AChE, TcAChE) and *B. tabaci* AChE1 (BtAChE1). In the substrate binding domain, black triangles indicate amino acid residues that have the same position in both sequences. Red and blue triangles indicate amino acid residues from *B. tabaci* and *T. californica* sequences, respectively. The amino acid residues forming the catalytic triad (active site) of BtAChE1 are S262, E388, and H501. The dagger (†) and double dagger (‡) symbols indicate numbering in TcAChE and BtAChE1 sequences, respectively. Sequence gaps were excluded from numbering.



Supplementary Information S3. Molecular phylogeny of AChE1 from *B. tabaci* alongside with some other insect species, constructed with the neighbor-joining method. Cluster reliability was assessed with the nonparametric bootstrapping (10000 iterations). Branch labels refer to species, common name, GenBank accession number; the status (R; resistant, S; susceptible) of the *B. tabaci* tested strains is indicated. The evolutionary distance among species is shown at the bottom of the figure. The tree was rooted with AChE1 of the tick *Rhipicephalus microplus* as an outgroup.

			??!!?!!	
	BAW19591		EAVGWGHTRAQIPEAIECLRKVNASVL	
	BAW19585		cpp	
	BAW19586		cF	
Bemisia tabaci	ABV45412	312	cp 3	38
Cyrtorhinus lividipennis	AHW29553	268	cpKeDaDTTd. 2	94
Camponotus floridanus	EFN61766	339	p.A.nNLr.V.Dl.kD.De. 3	65
Anopheles gambiae	CAD32684	410	cp.epSKLsD.VgkDph 4	36
Chilo suppressalis	AKL78859	363	hcp.S <mark>1</mark> kDMgpmkS.De. 3	89
Rhipicephalus microplus	AJA71270	306	t.LRAPDS1DtLDScErPEdI 3	32
Tetranychus urticae	ADK12685	358	Qc.s.sTRsvHMQSIpe 3	884
			1 1 1 1	
	BAW19592		PVVDGSFLDEMPSKWVATKNFKKTNIL	
Bemisia tabaci	ABV45412	355		881
Lygus hesperus	JAG37539	308	.IA.V.DL.QRnL.NRI 3	34
Pediculus h. capitis	BAF46106	354		80
Phlebotomus papatasi	AFP20868	429	s.QR <mark>aM</mark> gRD 4	55
Nasonia vitripennis	K7IU37	310		336

Supplementary Information S4. Occurrence of mutations in specific segments of AChE1-type acetylcholinesterases from a range of insects, ticks, and mites according to ESTHER database. Variations in certain amino acid residues (particularly the hollow and black boxed ones) are represented. Question marks (?) indicate the position where the mutation has occurred in the aligned sequence and was correlated to the insecticide resistance. Exclamation points (!) highlight the other mutations points that were not related to insecticide resistance. The analysis encompasses some selected insect AChE1s resistant to organophosphates and/or to carbamates. Numbering the amino acid residues in the selected *B. tabaci* sequence segments from the present study follows the grey-shaded *B. tabaci* resistant allele sequence (ABV45412.1) (Alon et al. 2008).

Alon M, Alon F, Nauen R, Morin S (2008) Organophosphates' resistance in the B-biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) is associated with a point mutation in an *ace1*-type acetylcholinesterase and overexpression of carboxylesterase. Insect Biochem Mol Biol 38:940–949. https://doi.org/10.1016/j.ibmb.2008.07.007













Supplementary Information S5. The average binding energies of the different organophosphate **(A-K)** and carbamate **(L-V)** pesticides in the wild type (WT) and in the eleven studied mutants (F392W, S369W, L370V, C316W, P317G, P317F, G251D, N252R, P253S, C329S, and L330T).