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(54) COMPOSITIONS AND METHODS TO CONTROL INSECT PESTS

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(52) **U.S. Cl.**

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(57) **ABSTRACT**

Methods and compositions are provided which employ a silencing element that, when ingested by a pest, such as a Coleopteran plant pest or a *Diabrotica* plant pest, decrease the expression of a target sequence in the pest. In specific embodiments, the decrease in expression of the target sequence controls the pest and thereby the methods and compositions are capable of limiting damage to a plant. The present invention provides various target polynucleotides set forth in any one of SEQ ID NOS: disclosed herein, (but not including the forward and reverse primers.) or active variants and fragments thereof, or complements thereof, wherein a decrease in expression of one or more of the sequences in the target pest controls the pest (i.e., has insecticidal activity). Further provided are silencing elements which when ingested by the pest decrease the level of the target polypeptide and thereby control the pest. In specific embodiments, the pest is *D. virgifera virgifera, D. barberi, D. virgifera zeae, D. speciosa, D. speciosa*, or *D. undecimpunctata howardi*. Plants, plant parts, bacteria and other host cells comprising the silencing elements or an active variant or fragment thereof of the invention are also provided.

Specification includes a Sequence Listing.

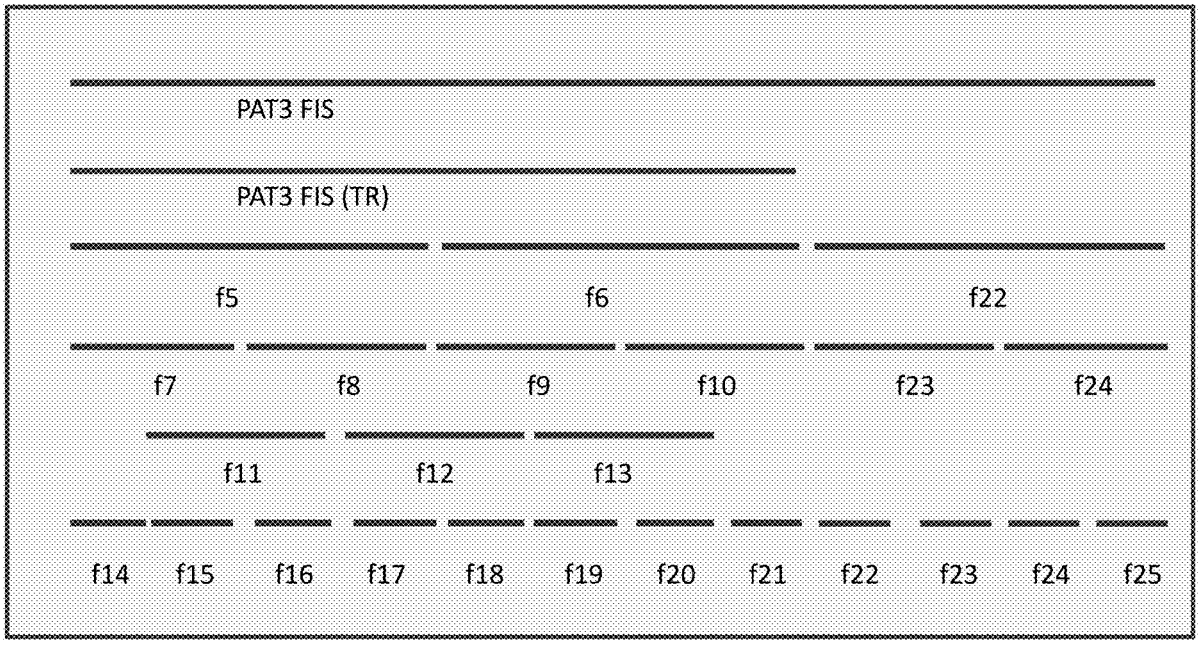


FIG. 1A

Table 1A

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
idv1c.pk037.j20.f	hypothetical protein	DV-HP2-FIS	Seq No.001	Seq No.002	Seq No.003	593	3
idv1c.pk032.n18.f:fis	RNA-dependent DNA polymerase	DV-POL-FIS	Seq No.005	Seq No.006	Seq No.007	446	1.5
idv1c.pk034.k22.f:fis	hypothetical protein TcasGA2_TC013063 [Tribolium castaneum]	DV-RNAPOL-FIS	Seq No.009	Seq No.010	Seq No.011	445	2.6
iwc1c.pk017.e6	Small GTPase superfamily, Rab 11		Seq No.013	Seq No.014	Seq No.015	563	2.9
iwc1c.pk019.o21	protein transport protein sec23	DV-PTP-FIS	Seq No.017	Seq No.018	Seq No.019	204	2.125
iwc1c.pk023.f12	putative elongation factor 1-alp		Seq No.021	Seq No.022	Seq No.023	599	1.5
iwc1c.pk026.d3	Vacuolar protein sorting-associated, VPS28		Seq No.025	Seq No.026	Seq No.027	550	2.9
iwc1c.pk026.e6	chaperonin subunit 6a zeta		Seq No.029	Seq No.030	Seq No.031	578	2.3
iwc1c.pk026.f16	nuclear lamin C protein		Seq No.033	Seq No.034	Seq No.035	709	1.7
iwc1c.pk026.h16	chaperonin	DV-CPNN-FIS	Seq No.037	Seq No.038	Seq No.039	635	1.8
iwc1c.pk027.i21	V-ATPase B subunit		Seq No.041	Seq No.042	Seq No.043	579	3.0
iwc1c.pk029.h21	translation elongation factor 2	DV-TEF2-FIS	Seq No.045	Seq No.046	Seq No.047	670	2.4
iwm2c.pk004.m2	signal recognition particle 54 kda protein	DV-SRP54-FIS	Seq No.049	Seq No.050	Seq No.051	528	2.6
iwm2c.pk005.j8	CG3612-PA isoform 2 [Tribolium castaneum]		Seq No.053	Seq No.054	Seq No.055	605	1.5
iwm2c.pk005.l7	chaperonin		Seq No.057	Seq No.058	Seq No.059	583	2.3
iwm2c.pk008.e24	translation initiation factor 3	DV-TIF3-FIS	Seq No.061	Seq No.062	Seq No.063	588	2.5
idv1c.pk015.b8.f:fis	GTP-binding protein SAR2	DV-BPSAR2-FIS	Seq No.065	Seq No.066	Seq No.067	716	3
idv1c.pk019.h19.f	homocysteine S-methyltransferase		Seq No.069	Seq No.070	Seq No.071	619	2.125

FIG. 1B

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
idv1c.pk019.h19.f	homocysteine S-methyltransferase		Seq No.069	Seq No.070	Seq No.071	619	2.125
idv1c.pk024.n1.f:fis	Transcription elongation factor SPT6-like protein		Seq No.073	Seq No.074	Seq No.075	680	2.8
idv1c.pk026.d10.f	Nucleosome Core, Chain C	DV-HP1-FIS	Seq No.077	Seq No.078	Seq No.079	319	2.625
idv1c.pk033.j21.f:fis	Proteasome subunit alpha type-6-like protein		Seq No.081	Seq No.082	Seq No.083	759	1.625
idv1c.pk037.j14.f	Ras-like GTP-binding protein Rho1	DV-RASRHO-FIS	Seq No.085	Seq No.086	Seq No.087	587	2.625
iwmlopc.pk023.i12.f	DNA-directed RNA polymerase II 13.3 kDa polypeptide		Seq No.089	Seq No.090	Seq No.091	444	2
idv1c.pk037.n13.f	Small GTPase superfamily, Ras type protein		Seq No.093	Seq No.094	Seq No.095	453	2.125
idv1c.pk038.b24.f	GTP-binding nuclear protein Ran		Seq No.097	Seq No.098	Seq No.099	601	2.25
idv1c.pk038.d14.f	DEAD box ATP-dependent RNA helicase	DV-DEAD-FIS	Seq No.101	Seq No.102	Seq No.103	614	2.625
idv1c.pk038.k10.f	ribosome-associated protein P40		Seq No.105	Seq No.106	Seq No.107	683	1.9
idv1c.pk038.p10.f	Arrest defective 1		Seq No.109	Seq No.110	Seq No.111	559	1.75
idv1c.pk040.c10.f	Nuclear transport factor 2 (NTF2) domain protein	DV-HPP15-FIS	Seq No.113	Seq No.114	Seq No.115	368	2.6
idv1c.pk040.j22.f	eukaryotic translation initiation factor		Seq No.117	Seq No.118	Seq No.119	589	2.25
idv1c.pk041.n22.f	Myosin heavy chain CG17927-PF isoform 1 [Tribolium castaneum]		Seq No.121	Seq No.122	Seq No.123	610	1.75
idv1c.pk042.g10.f	eukaryotic translation initiation factors		Seq No.125	Seq No.126	Seq No.127	628	2
idv1c.pk042.i20.f	AP-1 complex subunit mu-1-like isoform 1	DV-CAP-FIS	Seq No.129	Seq No.130	Seq No.131	619	2.13

FIG. 1C

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
idv1c.pk043.o11.f	Paramyosin, long form-like		Seq No.133	Seq No.134	Seq No.135	660	2.125
idv1c.pk002.j17.f:fis	preteasome 26S subunit, alpha type, 3	DV-PAT3-FIS	Seq No.137	Seq No.138	Seq No.139	589	2.43
idv1c.pk003.d6.f:fis	preteasome 26S subunit, beta type, 1	DV-PROTB-FIS	Seq No.141	Seq No.142	Seq No.143	577	2.25
idv1c.pk016.h19.f:fis	preteasome 26S subunit, beta type, 6 [9]	DV-PBT6-FIS	Seq No.145	Seq No.146	Seq No.147	542	2.63
idv1c.pk025.a4.f:fis	preteasome 26S subunit, non-ATPase, 3	DV-NATP3-FIS	Seq No.149	Seq No.150	Seq No.151	559	2.63
idv1c.pk033.j21.f:fis	preteasome 26S subunit, alpha type, 6	DV-PAT6-FIS	Seq No.153	Seq No.154	Seq No.155	473	1.50
idv1c.pk040.m14.f	preteasome 26S subunit, beta type, 3	DV-BETA3-FIS	Seq No.157	Seq No.158	Seq No.159	451	2.8
idv1c.pk046.m13.f	preteasome 26S subunit, non-ATPase, 14	DV-NATP14-FIS	Seq No.161	Seq No.162	Seq No.163	530	2.13
idv1c.pk047.d23.f	preteasome 26S subunit, alpha type, 1		Seq No.165	Seq No.166	Seq No.167	654	2.50
idv1c.pk047.i11.f	preteasome 26S subunit, beta type, 7 [10]	DV-BETA7-FIS	Seq No.169	Seq No.170	Seq No.171	558	2.00
idv1c.pk053.i16.f	preteasome 26S subunit, non-ATPase, 7	DV-NATP7-FIS	Seq No.173	Seq No.174	Seq No.175	473	2.25
idv1c.pk062.i5.f	preteasome 26S subunit, beta type, 4	DV-BETA4-FIS	Seq No.177	Seq No.178	Seq No.179	553	2.50
irw1c.pk010.o11.f	preteasome 26S subunit, non-ATPase, 8	DV-NATP8-FIS	Seq No.181	Seq No.182	Seq No.183	420	2.50
irw1c.pk011.c3.f	preteasome 26S subunit, beta type, 2	DV-BETA2-FIS	Seq No.185	Seq No.186	Seq No.187	716	2.38
iwc1c.pk003.n19	preteasome 26S subunit, non-ATPase, 2	DV-NATP2-FIS	Seq No.189	Seq No.190	Seq No.191	364	2.13
iwc1c.pk013.f20	preteasome 26S subunit, non-ATPase, 13		Seq No.193	Seq No.194	Seq No.195	358	1.83
iwc1c.pk018.g3	preteasome 26S subunit, ATPase, 1		Seq No.197	Seq No.198	Seq No.199	410	2.00
iwc1c.pk018.n9	preteasome 26S subunit, alpha type, 4		Seq No.201	Seq No.202	Seq No.203	458	2.50
iwc1c.pk022.l6	preteasome 26S subunit, ATPase, 5	DV-ATP5-FIS	Seq No.205	Seq No.206	Seq No.207	453	2.00

FIG. 1D

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
iwc1c.pk023.h8	preteasome 26S subunit, ATPase, 2		Seq No.209	Seq No.210	Seq No.211	488	2.00
iwc1c.pk028.o15	preteasome 26S subunit, non-ATPase, 11		Seq No.213	Seq No.214	Seq No.215	553	2.38
iwc1s.pk003.m17	preteasome 26S subunit, alpha type, 5	DV-PAT5-FIS	Seq No.217	Seq No.218	Seq No.219	469	2.38
iwm2c.pk009.f12	preteasome 26S subunit, ATPase, 4		Seq No.221	Seq No.222	Seq No.223	404	1.75
iwm2s.pk015.k15	preteasome 26S subunit, non-ATPase, 1	DV-NATP1-FIS	Seq No.225	Seq No.226	Seq No.227	455	2.25
454run3_isotig09801	Eukaryotic initiation factor 4A-like		Seq No.229	Seq No.230	Seq No.231	701	1.63
idv1c.pk044.p3.f	Eukaryotic initiation factor 4A-like		Seq No.233	Seq No.234	Seq No.235	669	1.5
idv1c.pk045.e5.f	Dosage compensation regulator maleless		Seq No.237	Seq No.238	Seq No.239	628	2.25
idv1c.pk045.h1.f	Phosphatidylinositol transfer protein alpha isoform-like protein		Seq No.241	Seq No.242	Seq No.243	610	1.625
idv1c.pk046.p17.f	small GTP binding protein RAB5		Seq No.245	Seq No.246	Seq No.247	524	2.375
idv1c.pk047.d23.f	proteasome subunit alpha		Seq No.249	Seq No.250	Seq No.251	529	2.375
idv1c.pk047.h18.f	Tetratricopeptide repeat protein 14-like		Seq No.253	Seq No.254	Seq No.255	533	2
idv1c.pk048.a20.f	mitochondrial cytochrome c1 [Tribolium castaneum]		Seq No.257	Seq No.258	Seq No.259	583	1.25
idv1c.pk048.c22.f	ubiquitin-activating enzyme E1 [Tribolium castaneum]		Seq No.261	Seq No.262	Seq No.263	546	2
idv1c.pk049.a20.f	Schizophyllum commune mitochondrial DNA, complete genome		Seq No.265	Seq No.266	Seq No.267	644	2
idv1c.pk049.b13.f	no significant hits		Seq No.269	Seq No.270	Seq No.271	629	1.5
idv1c.pk049.b16.f	Gelsolin repeat protein		Seq No.273	Seq No.274	Seq No.275	700	2

FIG. 1E

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
idv1c.pk049.b17.f	Glutathione S-transferase/chloride channel, C-terminal protein		Seq No.277	Seq No.278	Seq No.279	608	2
idv1c.pk049.i4.f	WD domain, G-beta repeat protein		Seq No.281	Seq No.282	Seq No.283	724	1.25
idv1c.pk049.m20.f	Zinc finger, DHHC-type, palmitoyltransferase		Seq No.285	Seq No.286	Seq No.287	501	1.625
idv1c.pk050.a13.f	Glutamate receptor, ionotropic ampa, subunit 1, 2, 3, 4		Seq No.289	Seq No.290	Seq No.291	628	2
idv1c.pk050.b3.f	Myosin binding subunit CG32156-PG [Tribolium castaneum]		Seq No.293	Seq No.294	Seq No.295	710	2
idv1c.pk053.p12.f	S5e ribosomal protein [Timarcha balearica]		Seq No.297	Seq No.298	Seq No.299	632	2
idv1c.pk054.k12.f	ribosomal protein L9e [Cicindela litorea]		Seq No.301	Seq No.302	Seq No.303	546	2.125
idv1c.pk057.e11.f	eukaryotic translation initiation factor 2 gamma subunit		Seq No.305	Seq No.306	Seq No.307	409	1.5
idv1c.pk057.h7.f	heat shock protein 70		Seq No.309	Seq No.310	Seq No.311	670	2.375
idv1c.pk057.n10.f	V-type proton ATPase subunit B		Seq No.313	Seq No.314	Seq No.315	672	2
idv1c.pk058.b17.f	Calmodulin 1 (phosphorylase kinase, delta)		Seq No.317	Seq No.318	Seq No.319	500	2.25
idv1c.pk058.i15.f	coatomer subunit beta [Tribolium castaneum]		Seq No.321	Seq No.322	Seq No.323	514	2.125
idv1c.pk058.p1.f	heat shock cognate 70		Seq No.325	Seq No.326	Seq No.327	606	2.25
idv1c.pk055.m8.f	Small GTPase superfamily, Ras type protein		Seq No.329	Seq No.330	Seq No.331	718	2.25
idv1c.pk060.g1.f	Homo sapiens PAC clone RP5-1007H16 from 7		Seq No.333	Seq No.334	Seq No.335	704	2

FIG. 1F

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
idv1c.pk060.g5.f	RNA-dependent DNA polymerase		Seq No.337	Seq No.338	Seq No.339	221	2.25
idv1c.pk062.o19	proteasome subunit alpha type 2		Seq No.341	Seq No.342	Seq No.343	656	2
idv3c.pk001.a13.f	acidic p0 ribosomal protein		Seq No.345	Seq No.346	Seq No.347	512	1.85
idv1c.pk062.d24.f	similar to serine palmitoyltransferase		Seq No.349	Seq No.350	Seq No.351	700	1.5
idv3c.pk007.i8.f	Cytoplasmic actin		Seq No.353	Seq No.354	Seq No.355	440	2.25
idv3c.pk008.h22.f	H+-ATPase V-type subunit		Seq No.357	Seq No.358	Seq No.359	600	2.125
idv3c.pk011.g2.f	similar to ribosomal protein L14		Seq No.361	Seq No.362	Seq No.363	525	2
idv3c.pk012.e23.f	similar to CG32019-PA [Tribolium castaneum]		Seq No.365	Seq No.366	Seq No.367	551	2
idv3c.pk013.g12.f	ubiquitin/ribosomal protein S27Ae fusion protein		Seq No.369	Seq No.370	Seq No.371	586	2
idv3c.pk016.a10.f	actin-depolymerizing factor 1 [Bombyx mori]		Seq No.373	Seq No.374	Seq No.375	611	2.125
idv3c.pk016.g12.f	putative ribosomal protein L17/23 [Diaphorina citri]		Seq No.377	Seq No.378	Seq No.379	357	2.125
idv3c.pk016.l10.f	Polyadenylate-binding protein		Seq No.381	Seq No.382	Seq No.383	520	1.75
idv3c.pk026.f22.f	MLE protein [Bombyx mori]		Seq No.385	Seq No.386	Seq No.387	490	1.625
iwmhipc.pk001.d23.f	actin		Seq No.389	Seq No.390	Seq No.391	658	2.625
iwmhipc.pk001.d24.f	unknown		Seq No.393	Seq No.394	Seq No.395	601	1.75
iwmhipc.pk003.i17.f	transport protein Sec61 subunit alpha 2		Seq No.397	Seq No.398	Seq No.399	600	2
iwmhipc.pk006.g5.f	ribosomal protein L6		Seq No.401	Seq No.402	Seq No.403	707	2.125
iwmhipc.pk005.i16.f	ribosomal protein S6 kinase beta-1-like isoform 1		Seq No.405	Seq No.406	Seq No.407	671	2.25

FIG. 1G

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
iwmhipc.pk003.o14.f	S5e ribosomal protein		Seq No.409	Seq No.410	Seq No.411	612	2.125
iwmhipc.pk004.b8.f	ribosomal protein L15e		Seq No.413	Seq No.414	Seq No.415	630	1.75
iwmhipc.pk004.d7.f	ribosomal protein L10e		Seq No.417	Seq No.418	Seq No.419	663	2
iwmhipc.pk006.o23.f	ribosomal protein L10Ae		Seq No.421	Seq No.422	Seq No.423	625	1.75
iwmhipc.pk011.g12.f	UPF0464 protein C15orf44 homolog [Nasonia vitripennis]		Seq No.425	Seq No.426	Seq No.427	693	2
iwmhipc.pk011.i2.f	Heat shock protein DnaJ		Seq No.429	Seq No.430	Seq No.431	651	1.875
iwmhipc.pk011.j6.f	nubbin [Tribolium castaneum]		Seq No.433	Seq No.434	Seq No.435	658	1.75
iwmhipc.pk011.j4.f	Conserved oligomeric Golgi complex, subunit 6		Seq No.437	Seq No.438	Seq No.439	647	1.625
iwmhipc.pk011.l17.f	Peptidase C2, calpain, large subunit		Seq No.441	Seq No.442	Seq No.443	711	1.625
iwmhipc.pk023.f4.f	cadherin-like gene		Seq No.445	Seq No.446	Seq No.447	579	2
iwmhipc.pk023.k22.f	Acetyl-coa acetyltransferase		Seq No.449	Seq No.450	Seq No.451	539	2.875
iwmhipc.pk023.l2.f	Cordon-bleu protein-like 1		Seq No.453	Seq No.454	Seq No.455	603	2
iwmhipc.pk023.l22.f	Prohibitin protein		Seq No.457	Seq No.458	Seq No.459	519	2.125
iwmhipc.pk026.a10.f	Multisynthetase complex, auxiliary protein		Seq No.461	Seq No.462	Seq No.463	602	2.625
iwmhipc.pk026.m2.f	KN motif and ankyrin repeat domain-containing protein 1-like protein		Seq No.465	Seq No.466	Seq No.467	508	2
iwmhipc.pk030.p16	ubiquitin B		Seq No.469	Seq No.470	Seq No.471	525	3
iwmhipc.pk030.l7.f	eukaryotic release factor 1 CG5605-PA		Seq No.473	Seq No.474	Seq No.475	653	2

FIG. 1H

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
iwmhipc.pk028.c16.f	vacuolar ATPase subunit C		Seq No.477	Seq No.478	Seq No.479	577	1.875
iwmhipc.pk031.d11.f	ribosomal protein L7		Seq No.481	Seq No.482	Seq No.483	689	1.875
iwmhipc.pk031.j10.f	V-type proton ATPase subunit e-like protein		Seq No.485	Seq No.486	Seq No.487	327	2
iwmhipc.pk032.j18.f	skd/vacuolar sorting		Seq No.489	Seq No.490	Seq No.491	526	2
iwmhipc.pk034.h8.f	Vacuolar proton pump subunit H		Seq No.493	Seq No.494	Seq No.495	621	2
iwmhipc.pk034.i20.f	Eukaryotic translation initiation factor 3 subunit G-like protein		Seq No.497	Seq No.498	Seq No.499	642	2
iwmhipc.pk036.f24.f	Coatomer protein complex subunit delta		Seq No.501	Seq No.502	Seq No.503	524	2.65
iwmhipc.pk039.j12.f	eukaryotic translation initiation factor 3 subunit C-like		Seq No.505	Seq No.506	Seq No.507	612	1.875
iwmhipc.pk039.k19.f	Cytochrome P450 CYP9Z1 (Cyp9z1)		Seq No.509	Seq No.510	Seq No.511	503	1.875
iwmhipc.pk040.n14.f	ribosomal protein S23-like protein		Seq No.513	Seq No.514	Seq No.515	502	2
iwmhipc.pk041.n17.f	Eukaryotic translation initiation factor 3 subunit 9		Seq No.517	Seq No.518	Seq No.519	719	2
iwmhipc.pk052.j3.f	ATP synthase alpha subunit vacuolar		Seq No.521	Seq No.522	Seq No.523	639	2
iwmlopc.pk002.f13.f	ribosomal protein L21		Seq No.525	Seq No.526	Seq No.527	507	2.125
iwmlopc.pk031.c23.f	ribosomal protein		Seq No.529	Seq No.530	Seq No.531	519	2
iwmlopc.pk038.m15.f	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit		Seq No.533	Seq No.534	Seq No.535	600	2.25
iwmlopc.pk042.g3.f	unknown		Seq No.537	Seq No.538	Seq No.539	612	1.875
iwmlopc.pk006.g13.f	60S ribosomal protein L23a-like		Seq No.541	Seq No.542	Seq No.543	558	2.25
iwmlopc.pk006.g16.f	ribosomal protein L18e		Seq No.545	Seq No.546	Seq No.547	544	2

FIG. 1I

cDNA ID	GENE ID	Target Name	Seq No. (RNAi target)	Seq No. (PCR forward primer)	Seq No. (PCR reverse primer)	RNA Length	Primary Score
iwmiopc.pk007.g1.f	ribosomal protein L35A		Seq No.549	Seq No.550	Seq No.551	401	2
iwmiopc.pk015.h20.f	ribosomal protein L12e		Seq No.553	Seq No.554	Seq No.555	542	2
iwmiopc.pk022.p14.f	ribosomal protein S11		Seq No.557	Seq No.558	Seq No.559	450	2

FIG. 1J

Table 1B

cDNA ID	GENE ID	Target Name	Seq No. (WCRW transcript sequences)	Transcript ID	Length
idv1c.pk037.j20.f	hypothetical protein	DV-HP2-FIS	Seq No.004	ta01240.010_diavv	5689
idv1c.pk032.n18.f:fis	RNA-dependent DNA polymerase	DV-POL-FIS	Seq No.008	ta01405.001_diavv	7388
idv1c.pk034.k22.f:fis	hypothetical protein TcasGA2_TC013063 [Tribolium castaneum]	DV-RNAPOL-FIS	Seq No.012	ta02059.001_diavv	544
iwc1c.pk017.e6	Small GTPase superfamily, Rab 11		Seq No.016	ta47051.001_diavv	689
iwc1c.pk019.o21	protein transport protein sec23	DV-PTP-FIS	Seq No.020	ta01733.001_diavv	2636
iwc1c.pk023.f12	putative elongation factor 1-alp		Seq No.024	ta04714.004_diavv	1698
iwc1c.pk026.d3	Vacuolar protein sorting-associated, VPS28		Seq No.028	ta01620.001_diavv	769
iwc1c.pk026.e6	chaperonin subunit 6a zeta		Seq No.032	ta00896.001_diavv	1864
iwc1c.pk026.f16	nuclear lamin C protein		Seq No.036	ta03354.001_diavv	2662
iwc1c.pk026.h16	chaperonin	DV-CPNN-FIS	Seq No.040	ta04788.001_diavv	1866
iwc1c.pk027.i21	V-ATPase B subunit		Seq No.044	ta00620.009_diavv	2471
iwc1c.pk029.h21	translation elongation factor 2	DV-TEF2-FIS	Seq No.048	ta02030.005_diavv	4535
iwm2c.pk004.m2	signal recognition particle 54 kda protein	DV-SRP54-FIS	Seq No.052	ta05283.001_diavv	1927
iwm2c.pk005.j8	CG3612-PA isoform 2 [Tribolium castaneum]		Seq No.056	ta00263.005_diavv	2003
iwm2c.pk005.l7	chaperonin		Seq No.060	ta05506.001_diavv	1889
iwm2c.pk008.e24	translation initiation factor 3	DV-TIF3-FIS	Seq No.064	ta05000.002_diavv	3734
idv1c.pk015.b8.f:fis	GTP-binding protein SAR2	DV-BPSAR2-FIS	Seq No.068	ta00303.001_diavv	2438
idv1c.pk019.h19.f	homocysteine S-methyltransferase		Seq No.072	ta08045.004_diavv	1246
idv1c.pk024.n1.f:fis	Transcription elongation factor SPT6-like protein		Seq No.076	ta02840.001_diavv	6054
idv1c.pk026.d10.f	Nucleosome Core, Chain C	DV-HP1-FIS	Seq No.080	ta07804.001_diavv	456
idv1c.pk033.j21.f:fis	Proteasome subunit alpha type-6-like protein		Seq No.084	ta04410.001_diavv	957
idv1c.pk037.j14.f	Ras-like GTP-binding protein Rho1	DV-RASRHO-FIS	Seq No.088	ta15897.001_diavv	1530
iwmlopc.pk023.i12.f	DNA-directed RNA polymerase II 13.3 kDa polypeptide		Seq No.092	ta06570.001_diavv	575

FIG. 1K

cDNA ID	GENE ID	Target Name	Seq No. (WCRW transcript sequences)	Transcript ID	Length
idv1c.pk037.n13.f	Small GTPase superfamily, Ras type protein		Seq No.096	ta40942.001_diavv	640
idv1c.pk038.b24.f	GTP-binding nuclear protein Ran		Seq No.100	ta05966.001_diavv	1075
idv1c.pk038.d14.f	DEAD box ATP-dependent RNA helicase	DV-DEAD-FIS	Seq No.104	ta03347.002_diavv	1515
idv1c.pk038.k10.f	ribosome-associated protein P40		Seq No.108	ta15911.001_diavv	822
idv1c.pk038.p10.f	Arrest defective 1		Seq No.112	ta05802.003_diavv	903
idv1c.pk040.c10.f	Nuclear transport factor 2 (NTF2) domain protein	DV-HPP15-FIS	Seq No.116	ta08838.001_diavv	470
idv1c.pk040.j22.f	eukaryotic translation initiation factor		Seq No.120	ta00006.001_diavv	1418
idv1c.pk041.n22.f	Myosin heavy chain CG17927-PF isoform 1 [Tribolium castaneum]		Seq No.124	ta41130.001_diavv	669
idv1c.pk042.g10.f	eukaryotic translation initiation factors		Seq No.128	ta05495.001_diavv	1547
idv1c.pk042.i20.f	AP-1 complex subunit mu-1-like isoform 1	DV-CAP-FIS	Seq No.132	ta08478.001_diavv	2131
idv1c.pk043.o11.f	Paramyosin, long form-like		Seq No.136	ta00254.002_diavv	3369
idv1c.pk002.j17.f:fis	preteasome 26S subunit, alpha type, 3	DV-PAT3-FIS	Seq No.140	ta06609.001_diavv	956
idv1c.pk003.d6.f:fis	preteasome 26S subunit, beta type, 1	DV-PROTB-FIS	Seq No.144	ta03835.001_diavv	913
idv1c.pk016.h19.f:fis	preteasome 26S subunit, beta type, 6 [9]	DV-PBT6-FIS	Seq No.148	ta02145.001_diavv	850
idv1c.pk025.a4.f:fis	preteasome 26S subunit, non-ATPase, 3	DV-NATP3-FIS	Seq No.152	ta08208.001_diavv	1711
idv1c.pk033.j21.f:fis	preteasome 26S subunit, alpha type, 6	DV-PAT6-FIS	Seq No.156	454run3_isotig15710	879
idv1c.pk040.m14.f	preteasome 26S subunit, beta type, 3	DV-BETA3-FIS	Seq No.160	ta03399.001_diavv	829
idv1c.pk046.m13.f	preteasome 26S subunit, non-ATPase, 14	DV-NATP14-FIS	Seq No.164	ta04924.001_diavv	1190
idv1c.pk047.d23.f	preteasome 26S subunit, alpha type, 1		Seq No.168	ta04466.001_diavv	1142
idv1c.pk047.i11.f	preteasome 26S subunit, beta type, 7 [10]	DV-BETA7-FIS	Seq No.172	ta04816.001_diavv	1578
idv1c.pk053.i16.f	preteasome 26S subunit, non-ATPase, 7	DV-NATP7-FIS	Seq No.176	ta03894.001_diavv	1221
idv1c.pk062.i5.f	preteasome 26S subunit, beta type, 4	DV-BETA4-FIS	Seq No.180	ta01874.001_diavv	925
irw1c.pk010.o11.f	preteasome 26S subunit, non-ATPase, 8	DV-NATP8-FIS	Seq No.184	ta06150.001_diavv	1040
irw1c.pk011.c3.f	preteasome 26S subunit, beta type, 2	DV-BETA2-FIS	Seq No.188	ta01941.001_diavv	872
iwc1c.pk003.n19	preteasome 26S subunit, non-ATPase, 2	DV-NATP2-FIS	Seq No.192	ta44362.001_diavv	3066

FIG. 1L

cDNA ID	GENE ID	Target Name	Seq No. (WCRW transcript sequences)	Transcript ID	Length
iwc1c.pk013.f20	preteasome 26S subunit, non-ATPase, 13		Seq No.196	ta04679.001_diavv	1241
iwc1c.pk018.g3	preteasome 26S subunit, ATPase, 1		Seq No.200	ta07921.001_diavv	1452
iwc1c.pk018.n9	preteasome 26S subunit, alpha type, 4		Seq No.204	ta04612.001_diavv	892
iwc1c.pk022.l6	preteasome 26S subunit, ATPase, 5	DV-ATP5-FIS	Seq No.208	ta01297.001_diavv	1322
iwc1c.pk023.h8	preteasome 26S subunit, ATPase, 2		Seq No.212	454run3_isotig16670	1425
iwc1c.pk028.o15	preteasome 26S subunit, non-ATPase, 11		Seq No.216	ta03917.001_diavv	1860
iwc1s.pk003.m17	preteasome 26S subunit, alpha type, 5	DV-PAT5-FIS	Seq No.220	ta09615.001_diavv	850
iwm2c.pk009.f12	preteasome 26S subunit, ATPase, 4		Seq No.224	ta03370.001_diavv	1364
iwm2s.pk015.k15	preteasome 26S subunit, non-ATPase, 1	DV-NATP1-FIS	Seq No.228	ta00483.001_diavv	3242
454run3_isotig09801	Eukaryotic initiation factor 4A-like		Seq No.232	ta03651.003_diavv	2338
idv1c.pk044.p3.f	Eukaryotic initiation factor 4A-like		Seq No.236	454run12_isotig05267	1764
idv1c.pk045.e5.f	Dosage compensation regulator maleless		Seq No.240	ta41281.001_diavv	697
idv1c.pk045.h1.f	Phosphatidylinositol transfer protein alpha isoform-like protein		Seq No.244	ta20868.001_diavv	800
idv1c.pk046.p17.f	small GTP binding protein RAB5		Seq No.248	ta01834.005_diavv	3182
idv1c.pk047.d23.f	proteasome subunit alpha		Seq No.252	454run3_isotig10716	1051
idv1c.pk047.h18.f	Tetratricopeptide repeat protein 14-like		Seq No.256	ta01498.002_diavv	3832
idv1c.pk048.a20.f	mitochondrial cytochrome c1 [Tribolium castaneum]		Seq No.260	ta01835.001_diavv	1332
idv1c.pk048.c22.f	ubiquitin-activating enzyme E1 [Tribolium castaneum]		Seq No.264	ta05794.001_diavv	4199
idv1c.pk049.a20.f	Schizophyllum commune mitochondrial DNA, complete genome		Seq No.268	ta41459.001_diavv	712
idv1c.pk049.b13.f	no significant hits		Seq No.272	ta03339.001_diavv	1750
idv1c.pk049.b16.f	Gelsolin repeat protein		Seq No.276	ta00090.001_diavv	2169
idv1c.pk049.b17.f	Glutathione S-transferase/chloride channel, C-terminal protein		Seq No.280	ta20625.001_diavv	1508
idv1c.pk049.i4.f	WD domain, G-beta repeat protein		Seq No.284	ta06970.001_diavv	1151

FIG. 1M

cDNA ID	GENE ID	Target Name	Seq No. (WCRW transcript sequences)	Transcript ID	Length
idv1c.pk049.m20.f	Zinc finger, DHHC-type, palmitoyltransferase		Seq No.288	ta06058.001_diavv	2197
idv1c.pk050.a13.f	Glutamate receptor, ionotropic ampa, subunit 1, 2, 3, 4		Seq No.292	ta41486.001_diavv	690
idv1c.pk050.b3.f	Myosin binding subunit CG32156-PG [Tribolium castaneum]		Seq No.296	ta00860.008_diavv	3435
idv1c.pk053.p12.f	S5e ribosomal protein [Timarcha balearica]		Seq No.300	ta21523.001_diavv	806
idv1c.pk054.k12.f	ribosomal protein L9e [Cicindela litorea]		Seq No.304	ta02181.002_diavv	789
idv1c.pk057.e11.f	eukaryotic translation initiation factor 2 gamma subunit		Seq No.308	ta02330.001_diavv	1691
idv1c.pk057.h7.f	heat shock protein 70		Seq No.312	ta00491.010_diavv	2609
idv1c.pk057.n10.f	V-type proton ATPase subunit B		Seq No.316	ta00620.001_diavv	2583
idv1c.pk058.b17.f	Calmodulin 1 (phosphorylase kinase, delta)		Seq No.320	ta02046.005_diavv	713
idv1c.pk058.i15.f	coatomer subunit beta [Tribolium castaneum]		Seq No.324	ta00934.001_diavv	3271
idv1c.pk058.p1.f	heat shock cognate 70		Seq No.328	454run12_isotig00560	2209
idv1c.pk055.m8.f	Small GTPase superfamily, Ras type protein		Seq No.332	ta00232.001_diavv	4464
idv1c.pk060.g1.f	Homo sapiens PAC clone RP5-1007H16 from 7		Seq No.336	ta41917.001_diavv	737
idv1c.pk060.g5.f	RNA-dependent DNA polymerase		Seq No.340	ta03247.005_diavv	3813
idv1c.pk062.o19	proteasome subunit alpha type 2		Seq No.344	ta03948.001_diavv	857
idv3c.pk001.a13.f	acidic p0 ribosomal protein		Seq No.348	ta20424.001_diavv	1042
idv1c.pk062.d24.f	similar to serine palmitoyltransferase		Seq No.352	ta00669.001_diavv	2113
idv3c.pk007.i8.f	Cytoplasmic actin		Seq No.356	ta15851.001_diavv	1465
idv3c.pk008.h22.f	H+-ATPase V-type subunit		Seq No.360	ta15854.001_diavv	2938
idv3c.pk011.g2.f	similar to ribosomal protein L14		Seq No.364	ta01695.001_diavv	689
idv3c.pk012.e23.f	similar to CG32019-PA [Tribolium castaneum]		Seq No.368	ta35139.001_diavv	6974
idv3c.pk013.g12.f	ubiquitin/ribosomal protein S27Ae fusion protein		Seq No.372	ta06472.004_diavv	683
idv3c.pk016.a10.f	actin-depolymerizing factor 1 [Bombyx mori]		Seq No.376	ta00957.001_diavv	1099
idv3c.pk016.g12.f	putative ribosomal protein L17/23 [Diaphorina citri]		Seq No.380	ta01259.001_diavv	667
idv3c.pk016.i10.f	Polyadenylate-binding protein		Seq No.384	ta16449.001_diavv	2656

FIG. 1N

cDNA ID	GENE ID	Target Name	Seq No. (WCRW transcript sequences)	Transcript ID	Length
idv3c.pk026.f22.f	MLE protein [Bombyx mori]		Seq No.388	ta01430.001_diavv	4007
iwmhipc.pk001.d23.f	actin		Seq No.392	ta02481.002_diavv	1534
iwmhipc.pk001.d24.f	unknown		Seq No.396	ta08003.008_diavv	1208
iwmhipc.pk003.i17.f	transport protein Sec61 subunit alpha 2		Seq No.400	ta03313.003_diavv	2110
iwmhipc.pk006.g5.f	ribosomal protein L6		Seq No.404	ta17558.001_diavv	1036
iwmhipc.pk005.i16.f	ribosomal protein S6 kinase beta-1-like isoform 1		Seq No.408	ta00443.001_diavv	2032
iwmhipc.pk003.o14.f	S5e ribosomal protein		Seq No.412	454run3_isotig03469	780
iwmhipc.pk004.b8.f	ribosomal protein L15e		Seq No.416	ta18374.001_diavv	1374
iwmhipc.pk004.d7.f	ribosomal protein L10e		Seq No.420	ta04337.003_diavv	726
iwmhipc.pk006.o23.f	ribosomal protein L10Ae		Seq No.424	ta02345.005_diavv	1055
iwmhipc.pk011.g12.f	UPF0464 protein C15orf44 homolog [Nasonia vitripennis]		Seq No.428	ta10911.001_diavv	1551
iwmhipc.pk011.i2.f	Heat shock protein DnaJ		Seq No.432	ta05539.003_diavv	1310
iwmhipc.pk011.j6.f	nubbin [Tribolium castaneum]		Seq No.436	ta21806.001_diavv	698
iwmhipc.pk011.j4.f	Conserved oligomeric Golgi complex, subunit 6		Seq No.440	ta21805.001_diavv	727
iwmhipc.pk011.l17.f	Peptidase C2, calpain, large subunit		Seq No.444	ta05594.001_diavv	2478
iwmhipc.pk023.f4.f	cadherin-like gene		Seq No.448	ta21896.001_diavv	628
iwmhipc.pk023.k22.f	Acetyl-coa acetyltransferase		Seq No.452	ta05758.001_diavv	1541
iwmhipc.pk023.l2.f	Cordon-bleu protein-like 1		Seq No.456	ta14242.001_diavv	736
iwmhipc.pk023.l22.f	Prohibitin protein		Seq No.460	ta06694.001_diavv	1170
iwmhipc.pk026.a10.f	Multisynthetase complex, auxiliary protein		Seq No.464	ta07588.001_diavv	1170
iwmhipc.pk026.m2.f	KN motif and ankyrin repeat domain-containing protein 1-like protein		Seq No.468	ta01520.001_diavv	3569
iwmhipc.pk030.p16	ubiquitin B		Seq No.472	ta21603.001_diavv	703
iwmhipc.pk030.l7.f	eukaryotic release factor 1 CG5605-PA		Seq No.476	ta00933.001_diavv	2829
iwmhipc.pk028.c16.f	vacuolar ATPase subunit C		Seq No.480	ta01215.004_diavv	2409
iwmhipc.pk031.d11.f	ribosomal protein L7		Seq No.484	ta05864.001_diavv	855
iwmhipc.pk031.j10.f	V-type proton ATPase subunit e-like protein		Seq No.488	ta06727.001_diavv	656

FIG. 10

cDNA ID	GENE ID	Target Name	Seq No. (WCRW transcript sequences)	Transcript ID	Length
iwmhipc.pk032.j18.f	skd/vacuolar sorting		Seq No.492	ta02217.001_diavv	2068
iwmhipc.pk034.h8.f	Vacuolar proton pump subunit H		Seq No.496	ta02168.002_diavv	1830
iwmhipc.pk034.i20.f	Eukaryotic translation initiation factor 3 subunit G-like protein		Seq No.500	ta06291.001_diavv	1014
iwmhipc.pk036.f24.f	Coatomer protein complex subunit delta		Seq No.504	ta03896.001_diavv	1812
iwmhipc.pk039.j12.f	eukaryotic translation initiation factor 3 subunit C-like		Seq No.508	ta05048.001_diavv	2785
iwmhipc.pk039.k19.f	Cytochrome P450 CYP9Z1 (Cyp9z1)		Seq No.512	ta01391.001_diavv	2131
iwmhipc.pk040.n14.f	ribosomal protein S23-like protein		Seq No.516	ta00545.001_diavv	616
iwmhipc.pk041.n17.f	Eukaryotic translation initiation factor 3 subunit 9		Seq No.520	ta01172.001_diavv	2397
iwmhipc.pk052.j3.f	ATP synthase alpha subunit vacuolar		Seq No.524	ta01291.009_diavv	2533
iwmlopc.pk002.f13.f	ribosomal protein L21		Seq No.528	ta01222.003_diavv	696
iwmlopc.pk031.c23.f	ribosomal protein		Seq No.532	ta05572.002_diavv	596
iwmlopc.pk038.m15.f	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit		Seq No.536	ta03508.001_diavv	797
iwmlopc.pk042.g3.f	unknown		Seq No.540	ta03675.001_diavv	893
iwmlopc.pk006.g13.f	60S ribosomal protein L23a-like		Seq No.544	ta00525.004_diavv	1017
iwmlopc.pk006.g16.f	ribosomal protein L18e		Seq No.548	ta05162.001_diavv	793
iwmlopc.pk007.g1.f	ribosomal protein L35A		Seq No.552	ta00344.005_diavv	1008
iwmlopc.pk015.h20.f	ribosomal protein L12e		Seq No.556	ta01789.001_diavv	768
iwmlopc.pk022.p14.f	ribosomal protein S11		Seq No.560	ta02086.002_diavv	615

FIG. 2A

Table 2

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-HP2-FIS	idv1c.pk037.j20.f	hypothetical protein	3	0.560	1.738	0.366	Seq No.001	593
DV-HP2-FRAG1	idv1c.pk037.j20.f	hypothetical protein	2.625	0.450	0.447	0.149	Seq No.561	225
DV-HP2-FRAG2	idv1c.pk037.j20.f	hypothetical protein	2.625	0.350	1.947	0.331	Seq No.562	212
DV-HP2-FRAG3	idv1c.pk037.j20.f	hypothetical protein	2.625	0.516	1.321	0.158	Seq No.563	210
DV-HP2-FRAG4	idv1c.pk037.j20.f	hypothetical protein	3	4.688			Seq No.564	229
DV-HP2-FRAG5	idv1c.pk037.j20.f	hypothetical protein	2.625	0.356	0.752	0.255	Seq No.565	170
DV-HP2-FRAG6	idv1c.pk037.j20.f	hypothetical protein	2.375				Seq No.566	233
DV-HP2-FRAG7	idv1c.pk037.j20.f	hypothetical protein	2.375	0.056	0.121	0.054	Seq No.567	162
DV-HP2-FRAG8	idv1c.pk037.j20.f	hypothetical protein	2.286	0.087	0.112	0.053	Seq No.568	473
DV-HP2-FRAG9	idv1c.pk037.j20.f	hypothetical protein	2.143	0.097	0.221	0.087	Seq No.569	430
DV-HP2-FRAG10	idv1c.pk037.j20.f	hypothetical protein	2	0.236	0.675	0.138	Seq No.570	486
DV-HP2-FRAG11	idv1c.pk037.j20.f	hypothetical protein	2.125	0.344	0.341	0.046	Seq No.571	500
DV-HP2-FRAG12	idv1c.pk037.j20.f	hypothetical protein	2.125	0.346	0.429	0.074	Seq No.572	310
DV-RYANR-FIS	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2.75	0.480			Seq No.573	1327
DV-RYANR-FRAG1	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2.875	0.277	0.045	0.014	Seq No.574	194
DV-RYANR-FRAG2	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	**3**	0.006	0.098	0.014	Seq No.575	145

FIG. 2B

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-RYANR-FRAG3	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2.875	0.004	0.036	0.011	Seq No.576	83
DV-RYANR-FRAG4	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2.375	0.062	0.044	0.015	Seq No.577	292
DV-RYANR-FRAG5	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2	0.013	0.163	0.300	Seq No.578	502
DV-RYANR-FRAG6	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	1.1429				Seq No.579	199
DV-RYANR-FRAG7	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	0.5				Seq No.580	197
DV-RYANR-FRAG8	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2.25	0.123			Seq No.581	142
DV-RYANR-FRAG9	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2.625	0.145	0.411	0.090	Seq No.582	129
DV-RYANR-FRAG10	idv1c.pk035.i17.f:fis	ryanodine receptor-like protein	2.375	0.336	0.492	0.126	Seq No.583	152
DV-RASRHO-FIS	idv1c.pk037.j14.f	Ras-like GTP-binding protein Rho1	2.625				Seq No.584	587
DV-RASRHO-FRAG1	idv1c.pk037.j14.f	Ras-like GTP-binding protein Rho2	2.3	3.860			Seq No.585	163
DV-DEAD-FIS	idv1c.pk038.d14.f	DEAD box ATP-dependent RNA helicase	2.625	no			Seq No.101	614
DV-DEAD-FRAG1	idv1c.pk038.d14.f	DEAD box ATP-dependent RNA helicase	2.25	51.509			Seq No.586	178
DV-HPP15-FIS	idv1c.pk040.c10.f	hypothetical protein-similar to p15-2a protein	2.6	31.170			Seq No.113	368
DV-HPP15-FRAG1	idv1c.pk040.c10.f	hypothetical protein-similar to p15-2a protein	1.4				Seq No.587	152
DV-BPSAR2-FIS	idv1c.pk015.b8.f:fis	GTP-binding protein SAR2	3	0.018	1.471	0.103	Seq No.065	716
DV-BPSAR2-FRAG1	idv1c.pk015.b8.f:fis	GTP-binding protein SAR2	2.75	0.532	1.306	0.154	Seq No.588	402
DV-BPSAR2-FRAG2	idv1c.pk015.b8.f:fis	GTP-binding protein SAR3	2.375	0.197	29.630	0.317	Seq No.589	171
DV-BPSAR2-FRAG3	idv1c.pk015.b8.f:fis	GTP-binding protein SAR4	2.125	6.481	1.907	0.230	Seq No.590	137

FIG. 2C

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-BPSAR2-FRAG3	idv1c.pk015.b8.f:fis	GTP-binding protein SAR4	2.125	6.481	1.907	0.230	Seq No.590	137
DV-BPSAR2-FRAG4	idv1c.pk015.b8.f:fis	GTP-binding protein SAR5	2.375				Seq No.591	135
DV-BPSAR2-FRAG5	idv1c.pk015.b8.f:fis	GTP-binding protein SAR2	0.75				Seq No.592	294
DV-BPSAR2-FRAG6	idv1c.pk015.b8.f:fis	GTP-binding protein SAR2	0.625				Seq No.593	407
DV-HP1-FIS	idv1c.pk026.d10.f	Nucleosome Core, Chain C	2.625	5.470			Seq No.077	319
DV-HP1-FRAG1	idv1c.pk026.d10.f	hypothetical protein	2.5	0.911	6.501	0.797	Seq No.594	168
DV-HP1-FRAG2	idv1c.pk026.d10.f	hypothetical protein	2.375				Seq No.595	190
DV-HP1-FRAG3	idv1c.pk026.d10.f	hypothetical protein	2.75	4.217			Seq No.596	159
DV-RNAPOL-FIS	idv1c.pk034.k22.f:fis	DNA directed RNA polymerase I	2.6	3.068			Seq No.009	445
DV-RNAPOL-FRAG1	idv1c.pk034.k22.f:fis	DNA directed RNA polymerase I	1.8				Seq No.597	196
DV-CPNN-FIS	iwc1c.pk026.h16	chaperonin	1.8	12.600			Seq No.037	635
DV-CPNN-FRAG1	iwc1c.pk026.h16	chaperonin	0.5				Seq No.598	163
DV-CPNN-FRAG2	iwc1c.pk026.h16	chaperonin	1.3				Seq No.599	236
DV-CPNN-FRAG3	iwc1c.pk026.h16	chaperonin	1.1				Seq No.600	149
DV-CPNN-FRAG4	iwc1c.pk026.h16	chaperonin	2.1				Seq No.601	194
DV-TEF2-FIS	iwc1c.pk029.h21	translation elongation factor 2	2.4				Seq No.045	670
DV-TEF2-FRAG1	iwc1c.pk029.h21	translation elongation factor 2	2.1	2.880	0.832	0.064	Seq No.602	785
DV-SRP54-FIS	iwm2c.pk004.m2	signal recognition particle 54 kda protein	2.6				Seq No.049	528

FIG. 2D

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-SRP54-FRAG1	iwm2c.pk004.m2	signal recognition particle 54 kda protein	2.125				Seq No.603	144
DV-SRP54-FRAG2	iwm2c.pk004.m2	signal recognition particle 54 kda protein	1.75				Seq No.604	364
DV-SRP54-FRAG3	iwm2c.pk004.m2	signal recognition particle 54 kda protein	1.75	8.900	100.000	8.235	Seq No.605	226
DV-SRP54-FRAG4	iwm2c.pk004.m2	signal recognition particle 54 kda protein	2.125				Seq No.606	163
DV-TIF3-FIS	iwm2c.pk008.e24	translation initiation factor 3	2.5	174.500			Seq No.061	588
DV-TIF3-FRAG1	iwm2c.pk008.e24	translation initiation factor 3	1.3				Seq No.607	171
DV-TIF3-FRAG2	iwm2c.pk008.e24	translation initiation factor 3	1.625				Seq No.608	232
DV-TIF3-FRAG3	iwm2c.pk008.e24	translation initiation factor 3	1.3				Seq No.609	166
DV-TIF3-FRAG4	iwm2c.pk008.e24	translation initiation factor 3	0.9				Seq No.610	239
DV-TIF3-FRAG5	iwm2c.pk008.e24	translation initiation factor 3	1.4				Seq No.611	227
DV-TIF3-FRAG6	iwm2c.pk008.e24	translation initiation factor 3	0.9				Seq No.612	224
DV-TIF3-FRAG7	iwm2c.pk008.e24	translation initiation factor 3	1.1				Seq No.613	154
DV-TIF3-FRAG8	iwm2c.pk008.e24	translation initiation factor 3	2.3	15.700			Seq No.614	742
DV-TIF3-FRAG9	iwm2c.pk008.e24	translation initiation factor 3	2.0	104.140			Seq No.615	619
DV-EIF-4A-FIS	idv1c.pk044.p3.f	Eukaryotic initiation factor 4A-like	1.5				Seq No.233	669
DV-EIF-4A-Frag 2	idv1c.pk044.p3.f	Eukaryotic initiation factor 4A-like	2.25	0.046	0.325	0.088	Seq No.616	713
DV-EIF-4A-FRAG3	idv1c.pk044.p3.f	Eukaryotic initiation factor 4A-like	2	0.579			Seq No.617	200

FIG. 2E

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-PAT3-FIS	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.43				Seq No.137	589
DV-PAT3-SS1	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	1.5	7.089	0.664	0.091	Seq No.618	270
DV-PAT3-FRAG5	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	2.000	27.980	0.992	Seq No.619	322
DV-PAT3-FRAG6	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	3.000			Seq No.620	322
DV-PAT3-FRAG7	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	2.000	90.480	3.526	Seq No.621	161
DV-PAT3-FRAG8	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	18.500	1.995	0.223	Seq No.622	161
DV-PAT3-FRAG9	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	8.300	11.430	0.269	Seq No.623	161
DV-PAT3-FRAG10	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	8.200	88.860	0.380	Seq No.624	161
DV-PAT3-FRAG11	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2	5.930			Seq No.625	162
DV-PAT3-FRAG12	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2	5.700	0.200		Seq No.626	160
DV-PAT3-FRAG13	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2	0.010	0.541	0.023	Seq No.627	161
DV-PAT3-FRAG14	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	1				Seq No.628	80
DV-PAT3-FRAG15	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	1.25				Seq No.629	81
DV-PAT3-FRAG16	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.25	2.820			Seq No.630	80
DV-PAT3-FRAG17	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	1				Seq No.631	81
DV-PAT3-FRAG18	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2	8.317	56.730	8.084	Seq No.632	80
DV-PAT3-FRAG19	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2	4.714	40.030	3.628	Seq No.633	81

FIG. 2F

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-PAT3-FRAG20	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	1	6.220	0.522	0.074	Seq No.634	80
DV-PAT3-FRAG21	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	1	45.000	83.610	0.502	Seq No.635	81
DV-PAT3-FIS (TR)	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	2.000	25.800	0.333	Seq No.636	644
DV-PAT3-FRAG22	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	23.200			Seq No.637	367
DV-PAT3-FRAG23	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	2.5	11.400			Seq No.638	182
DV-PAT3-FRAG24	idv1c.pk002.j17.f:fis	proteosome subunit alpha type 3	0				Seq No.639	185
DV-PAT5-FIS	iwc1s.pk003.m17	proteosome subunit alpha type, 5	2.38				Seq No.217	469
DV-PAT5-Frag1	iwc1s.pk003.m17	proteosome subunit alpha type, 5	1.43				Seq No.640	256
DV-PAT6-FIS	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	2.625				Seq No.153	473
DV-PAT6-FRAG1	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	2.125				Seq No.641	200
DV-PAT6-FRAG2	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	1.875				Seq No.642	216
DV-PAT6-FRAG3	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	1.875				Seq No.643	200
DV-PAT6-FRAG4	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	2	0.722	200.000	200.000	Seq No.644	195
DV-PAT6-FRAG5	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	1.625	2.265	2.105	0.266	Seq No.645	199
DV-PAT6-FRAG6	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	2.25	2.405	29.790	1.865	Seq No.646	165
DV-PAT6-FRAG7	idv1c.pk033.j21.f:fis	proteasome subunit alpha type 6	1.67	1.784	0.853	0.089	Seq No.647	157
DV-BETA2-FIS	irw1c.pk011.c3.f	proteasome subunit beta type, 2	2.38	0.346	3.826	0.089	Seq No.185	716

FIG. 2G

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-BETA2-FRAG1	irw1c.pk011.c3.f	proteasome subunit beta type, 2	2.00	5.750			Seq No.648	196
DV-BETA2-FRAG2	irw1c.pk011.c3.f	proteasome subunit beta type, 2	1.13				Seq No.649	187
DV-BETA2-FRAG3	irw1c.pk011.c3.f	proteasome subunit beta type, 2	2	0.492			Seq No.650	155
DV-BETA3-FIS	idv1c.pk040.m14.f	proteasome subunit beta type, 3	2.8				Seq No.157	451
DV-BETA3-FRAG1	idv1c.pk040.m14.f	proteasome subunit beta type, 3	2.125	2.270	4.533	0.468	Seq No.651	174
DV-PROTB-FIS	idv1c.pk003.d6.f:fis	Proteosome subunit beta type 1	2.25	0.010	4.105	0.186	Seq No.141	577
DV-PROTB-FRAG1	idv1c.pk003.d6.f:fis	Proteosome subunit beta type 1	2.125	0.198	4.558	0.047	Seq No.652	203
DV-PBT6.2-FIS	idv1c.pk016.h19.f:fis	Proteosome subunit beta type 6	2.63				Seq No.145	542
DV-PBT6.2-FRAG1	idv1c.pk016.h19.f:fis	Proteosome subunit beta type 6	2	0.108			Seq No.653	291
DV-PBT6.2-FRAG2	idv1c.pk016.h19.f:fis	Proteosome subunit beta type 6	2.6	0.071			Seq No.654	277
DV-PBT6.2-FRAG3	idv1c.pk016.h19.f:fis	Proteosome subunit beta type 6	2.6				Seq No.655	257
DV-PBT6.2-FRAG4	idv1c.pk016.h19.f:fis	Proteosome subunit beta type 6	2				Seq No.656	273
DV-PBT6.2-FRAG5	idv1c.pk016.h19.f:fis	Proteosome subunit beta type 6	2.125	0.479			Seq No.657	219
DV-PBT6.2-FRAG6	idv1c.pk016.h19.f:fis	Proteosome subunit beta type 6	2.75	0.068	11.940	0.047	Seq No.658	151
DV-BETA7-FIS	idv1c.pk047.i11.f	Proteosome subunit beta type 7	2.00				Seq No.169	558
DV-BETA7-FRAG1	idv1c.pk047.i11.f	Proteosome subunit beta type 7	1.63				Seq No.659	229
DV-BETA7-FRAG2	idv1c.pk047.i11.f	Proteosome subunit beta type 7	2	14.040			Seq No.660	285

FIG. 2H

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-BETA7-FRAG3	idv1c.pk047.i11.f	Proteosome subunit beta type 7	2.00				Seq No.661	188
DV-BETA7-FRAG4	idv1c.pk047.i11.f	Proteosome subunit beta type 7	2.00	26.580			Seq No.662	150
DV-BETA7-FRAG5	idv1c.pk047.i11.f	Proteosome subunit beta type 7	2.25	38.900			Seq No.663	168
DV-BETA7-FRAG6	idv1c.pk047.i11.f	Proteosome subunit beta type 7	2.13	24.990			Seq No.664	156
DV-NATP1-FIS	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.25				Seq No.225	455
DV-NATP1-FRAG1	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.25	0.435	3.806	0.192	Seq No.665	220
DV-NATP1-FRAG2	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.00				Seq No.666	222
DV-NATP1-FRAG3	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.00				Seq No.667	204
DV-NATP1-FRAG4	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.13				Seq No.668	155
DV-NATP1-FRAG5	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.00				Seq No.669	226
DV-NATP1-FRAG6	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.28				Seq No.670	205
DV-NATP1-FRAG7	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.25				Seq No.671	236
DV-NATP1-FRAG8	iwm2s.pk015.k15	Proteosome 26S subunit, non-ATPase, 1	2.00	0.142	4.590	0.120	Seq No.672	200
DV-NATP2-FIS	iwc1c.pk003.n19	Proteosome 26S subunit, non-ATPase, 2	2.25				Seq No.189	364
DV-NATP2-FRAG1	iwc1c.pk003.n19	Proteosome 26S subunit, non-ATPase, 2	0.25				Seq No.673	199
DV-NATP2-FRAG2	iwc1c.pk003.n19	Proteosome 26S subunit, non-ATPase, 2	0.375				Seq No.674	198
DV-NATP2-FRAG3	iwc1c.pk003.n19	Proteosome 26S subunit, non-ATPase, 2	0				Seq No.675	190

FIG. 2I

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-NATP2-FRAG4	iwc1c.pk003.n19	Proteosome 26S subunit, non-ATPase, 2	0				Seq No.676	223
DV-NATP2-FRAG5	iwc1c.pk003.n19	Proteosome 26S subunit, non-ATPase, 2	2.125				Seq No.677	165
DV-NATP2-FRAG6	iwc1c.pk003.n19	Proteosome 26S subunit, non-ATPase, 2	1.625				Seq No.678	156
DV-NATP3-FIS	idv1c.pk025.a4.f:fis	Proteosome 26S subunit, non-ATPase, 3	2.63	0.209	2.715	0.236	Seq No.149	559
DV-NATP3-FRAG1	idv1c.pk025.a4.f:fis	Proteosome 26S subunit, non-ATPase, 3	2.4	21.470			Seq No.679	222
DV-NATP3-FRAG2	idv1c.pk025.a4.f:fis	Proteosome 26S subunit, non-ATPase, 3	2.13	38.736			Seq No.680	222
DV-NATP3-FRAG3	idv1c.pk025.a4.f:fis	Proteosome 26S subunit, non-ATPase, 3	2.00	0.515.3	33.680	0.889	Seq No.681	159
DV-NATP7-FIS	idv1c.pk053.i16.f	Proteosome 26S subunit, non-ATPase, 7	2.25	0.280	2.105	0.735	Seq No.173	473
DV-NATP7-FRAG1	idv1c.pk053.i16.f	Proteosome 26S subunit, non-ATPase, 7	1.8				Seq No.682	161
DV-NATP8-FIS	irw1c.pk010.o11.f	Proteosome 26S subunit, non-ATPase, 8	2.50				Seq No.181	420
DV-NATP8-FRAG1	irw1c.pk010.o11.f	Proteosome 26S subunit, non-ATPase, 8	2.00				Seq No.683	151
DV-NATP8-FRAG2	irw1c.pk010.o11.f	Proteosome 26S subunit, non-ATPase, 8	2.00				Seq No.684	163
DV-NATP8-FRAG3	irw1c.pk010.o11.f	Proteosome 26S subunit, non-ATPase, 8	2.13	0.390	11.607	0.193	Seq No.685	172
DV-NATP14-FIS	idv1c.pk046.m13.f	Proteasome 26S subunit, non-ATPase, 14	2.13	0.443	0.623	0.167	Seq No.686	673
DV-NATP14-FRAG1	idv1c.pk046.m13.f	Proteasome 26S subunit, non-ATPase, 14	0.00				Seq No.687	246
DV-ATP5-FIS	iwc1c.pk022.l6	Proteasome 26S subunit, ATPase, 5	2.00				Seq No.205	453
DV-ATP5-FRAG1	iwc1c.pk022.l6	Proteasome 26S subunit, ATPase, 5	2	1.405	3.008	0.133	Seq No.688	205

FIG. 2J

Target Name	**Initial SEQ ID**	Frag GENE ID	**primary assay**	Informal IC50	Formal LC50	Formal IC50	**Seq No.**	**Length (bp)**
DV-ATP5-FRAG2	iwc1c.pk022.l6	Proteasome 26S subunit, ATPase, 5	2	0.746	0.550	0.116	Seq No.689	219
DV-ATP5-FRAG3	iwc1c.pk022.l6	Proteasome 26S subunit, ATPase, 5	2	4.428			Seq No.690	244
DV-ATP5-FRAG4	iwc1c.pk022.l6	Proteasome 26S subunit, ATPase, 5	2.125	17.218			Seq No.691	163
DV-ATP5-FRAG5	iwc1c.pk022.l6	Proteasome 26S subunit, ATPase, 5	0.00				Seq No.692	176

FIG. 3A

Table 3

Target	Insect sources	Target group	Transcript ID	Seq No.	length
Ryanr-Ssk	WCRW	target pest	ta00611.03_diavv	Seq No.693	1432
Ryanr-Ssk	SCRW	target pest	ta01434.01_diaun	Seq No.694	730
Ryanr-Ssk	NCRW	target pest	ta01092.02_diab	Seq No.695	1629
Ryanr-Ssk	MBB	expanded pest	ta26722.002_epiva	Seq No.696	720
Ryanr-Ssk	CPB	expanded pest	ta37400.0001_lepde	Seq No.697	1043
Ryanr-Ssk	Orius	no target insect	ta01487.01_oriin	Seq No.698	648
Ryanr-Ssk	CMAC	no target insect	ta21166.0001_vibdu	Seq No.699	820
HP2-mesh	WCRW	target pest	ta01240.10_diavv	Seq No. 004	5689
HP2-mesh	SCRW	target pest	ta00809.07_diaun	Seq No.700	5238
HP2-mesh	NCRW	target pest	ta06241.06_diab	Seq No.701	4313
HP2-mesh	MBB	expanded pest	ta29117.001_epiva	Seq No.702	1564
HP2-mesh	CPB	expanded pest	ta36833.0009_lepde	Seq No.703	5020
HP2-mesh	Orius	no target insect	ta02139.06_oriin	Seq No.704	3724
HP2-mesh	CMAC	no target insect	ta22533.0010_vibdu	Seq No.705	3845

FIG. 3B

Target	Insect sources	Target group	Transcript ID	Seq No.	length
PAT3	WCRW	target pest	ta06609.01_diavv	Seq No. 140	956
PAT3	SCRW	target pest	ta01950.01_diaun	Seq No.706	982
PAT3	NCRW	target pest	ta01738.01_diab	Seq No.707	795
PAT3	MBB	expanded pest	ta26097.001_epiva	Seq No.708	993
PAT3	CPB	expanded pest	ta37784.0003_lepde	Seq No.709	936
PAT3	Orius	no target insect	ta09672.01_oriin	Seq No.710	927
PAT3	CMAC	no target insect	ta36121.0001_vibdu	Seq No.711	1115
PROTB	WCRW	target pest	ta03835.01_diavv	Seq No.144	913
PROTB	SCRW	target pest	ta07256.01_diaun	Seq No.712	853
PROTB	NCRW	target pest	ta07284.01_diab	Seq No.713	856
PROTB	MBB	expanded pest	ta27792.001_epiva	Seq No.714	796
PROTB	CPB	expanded pest	ta37916.0001_lepde	Seq No.715	842
PROTB	Orius	no target insect	ta09310.01_oriin	Seq No.716	755
PROTB	CMAC	no target insect	ta23917.0001_vibdu	Seq No.717	813
PBT6	WCRW	target pest	ta02145.01_diavv	Seq No.148	850
PBT6	SCRW	target pest	ta08127.01_diaun	Seq No.718	785

FIG. 3C

Target	Insect sources	Target group	Transcript ID	Seq No.	length
PBT6	NCRW	target pest	ta05581.01_diab	Seq No.719	842
PBT6	MBB	expanded pest	ta23856.001_epiva	Seq No.720	935
PBT6	CPB	expanded pest	ta33564.0001_lepde	Seq No.721	1264
PBT6	Orius	no target insect	ta09100.01_oriin	Seq No.722	912
PBT6	CMAC	no target insect	ta16980.0001_vibdu	Seq No.723	686

FIG. 4

Amino acid sequence alignment of WCRW Ryanr and Drosophila Ssk

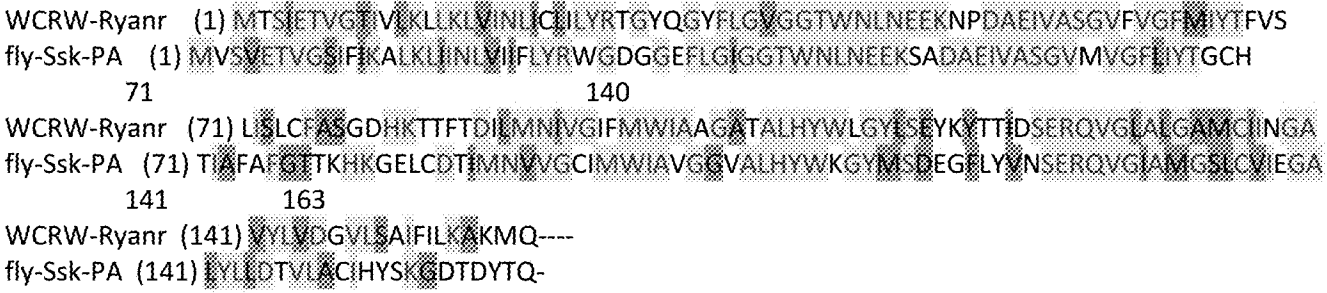
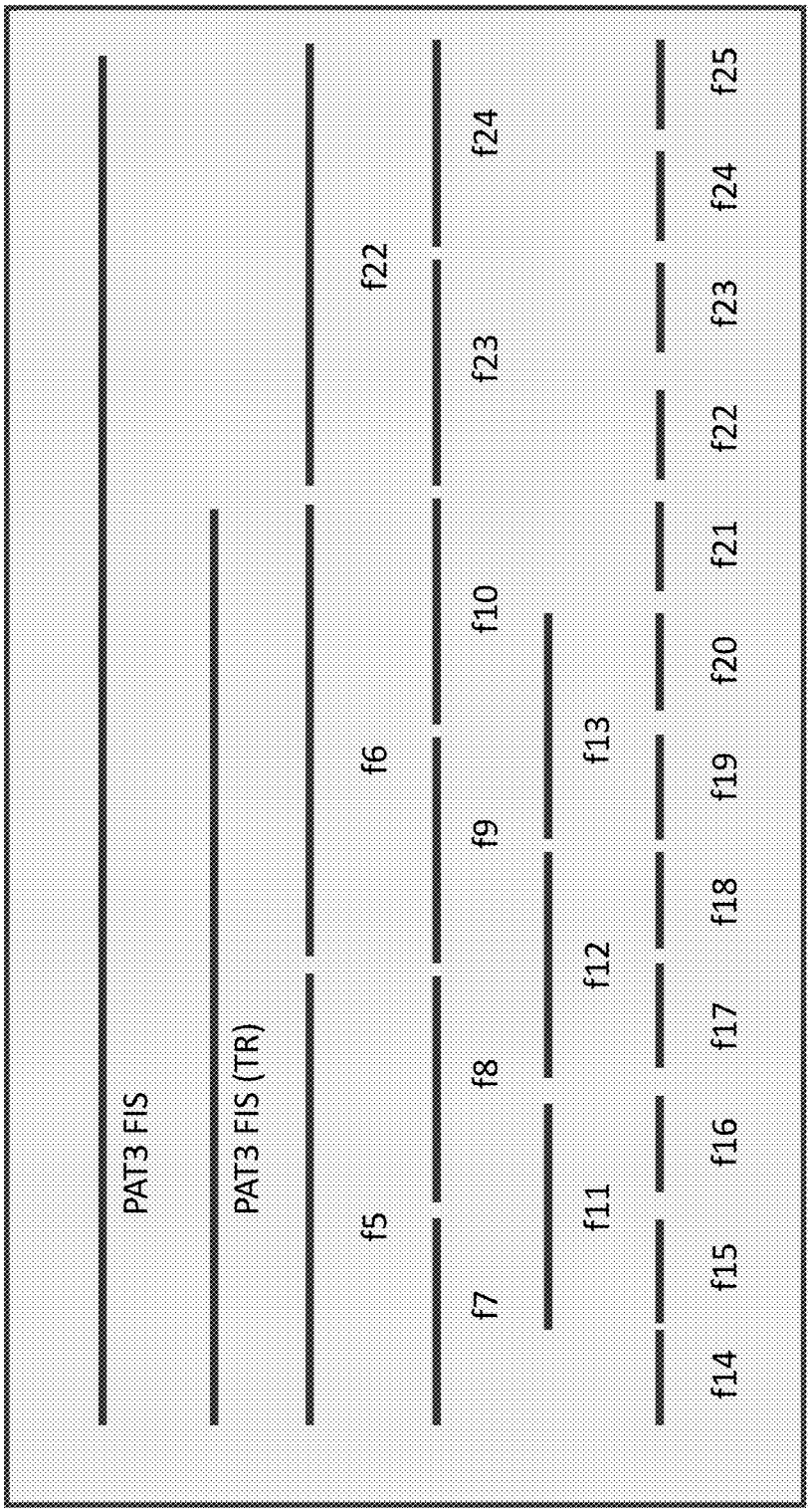


FIG. 5


PAT3 FIS
PAT3 FIS (TR)
f5
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f16
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COMPOSITIONS AND METHODS TO CONTROL INSECT PESTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. patent application Ser. No. 15/691,243, filed Aug. 30, 2017, which is a divisional of U.S. Utility application Ser. No. 13/831,230, filed Mar. 14, 2013, now U.S. Pat. No. 9,920,316, the disclosures of each of which are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates generally to methods of molecular biology and gene silencing to control pests.

REFERENCE TO A SEQUENCE LISTING

[0003] The official copy of the sequence listing is submitted electronically via USPTO Patent Center as an XML formatted sequence listing with a file named 5413USDIV2SequenceListing3 created on Sep. 24, 2023, and having a size of 1,440,066 bytes and is filed concurrently with the specification. The sequence listing comprised in this XML formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] Insect pests are a serious problem in agriculture. They destroy millions of acres of staple crops such as corn, soybeans, peas, and cotton. Yearly, these pests cause over $100 billion dollars in crop damage in the U.S. alone. In an ongoing seasonal battle, farmers must apply billions of gallons of synthetic pesticides to combat these pests. Other methods employed in the past delivered insecticidal activity by microorganisms or genes derived from microorganisms expressed in transgenic plants. For example, certain species of microorganisms of the genus *Bacillus* are known to possess pesticidal activity against a broad range of insect pests including Lepidoptera, Diptera, Coleoptera, Hemiptera, and others. In fact, microbial pesticides, particularly those obtained from *Bacillus* strains, have played an important role in agriculture as alternatives to chemical pest control. Agricultural scientists have developed crop plants with enhanced insect resistance by genetically engineering crop plants to produce insecticidal proteins from *Bacillus*. For example, corn and cotton plants genetically engineered to produce Cry toxins (see, e.g., Aronson (2002) *Cell Mol. Life Sci.* 59(3):417-425; Schnepf et al. (1998) *Microbiol. Mol. Biol. Rev.* 62(3):775-806) are now widely used in American agriculture and have provided the farmer with an alternative to traditional insect-control methods. However, these Bt insecticidal proteins only protect plants from a relatively narrow range of pests. Moreover, these modes of insecticidal activity provided varying levels of specificity and, in some cases, caused significant environmental consequences. Thus, there is an immediate need for alternative methods to control pests.

BRIEF SUMMARY OF THE INVENTION

[0005] Methods and compositions are provided which employ a silencing element that, when ingested by a pest, such as Coleopteran plant pest including a *Diabrotica* plant pest, is capable of decreasing the expression of a target sequence in the pest. In specific embodiments, the decrease in expression of the target sequence controls the pest and thereby the methods and compositions are capable of limiting damage to a plant. The present invention provides various target polynucleotides as set forth in SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants or fragments thereof, or complements thereof, wherein a decrease in expression of one or more of the sequences in the target pest controls the pest (i.e., has insecticidal activity). Further provided are silencing elements, which when ingested by the pest, decrease the level of expression of one or more of the target polynucleotides. Plants, plant parts, plant cells, bacteria and other host cells comprising the silencing elements or an active variant or fragment thereof are also provided. Also provided are formulations of sprayable silencing agents for topical applications to pest insects or substrates where pest insects may be found.

[0006] In another embodiment, a method for controlling a pest, such as a Coleopteran plant pest or a *Diabrotica* plant pest, is provided. The method comprises feeding to a pest a composition comprising a silencing element, wherein the silencing element, when ingested by the pest, reduces the level of a target sequence in the pest and thereby controls the pest. Further provided are methods to protect a plant from a pest. Such methods comprise introducing into the plant or plant part a silencing element of the invention. When the

plant expressing the silencing element is ingested by the pest, the level of the target sequence is decreased and the pest is controlled.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIGS. **1**A-**10** are tables, Tables 1A and 1B, which identifies RNAi active targets in diet assay using dsRNA produced by in vitro transcription (IVT).

[0008] FIGS. **2**A-**2**J are a table, Table 2, which shows design and identification of RNAi active fragments

[0009] FIGS. **3**A-**3**C are a table, Table 3, which lists RNAi active targets from target pests, expanded pests and no target insects. Homologous sequences of selected RNAi actives were identified from transcriptome analyses of Western corn rootworm (WCRW, *Diabrotica virgifera*), Northern corn rootworm (NCRW, *Diabrotica barberi*), Southern corn rootworm (SCRW, *Diabrotica undecimpunctata*), Mexican Bean Beetle (MBB, *Epilachna varivestis*), Colorado potato beetle (CPB, *Leptinotarsa decemlineata*), insidious flower bug (Orius, *Orius insidiosus*) and Spotted Lady Beetle (CMAC, *Coleomegilla maculate*).

[0010] FIG. **4** is a graphic showing a sequence alignment of the amino acid sequences of WCRW Ryanr (SEQ ID NO: 724) and *Drosophila* Ssk (SEQ ID NO: 725).

[0011] FIG. **5** is a schematic of PAT3 fragments used in the gene and construct optimization experiment.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present inventions now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the inventions are shown. Indeed, these inventions may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout.

[0013] Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

I. Overview

[0014] Frequently, RNAi discovery methods rely on evaluation of known classes of sensitive genes (transcription factors, housekeeping genes etc.). In contrast, the target polynucleotides set forth herein were identified based solely on high throughput screens of all singletons and representatives of all gene clusters from a cDNA library of neonate and/or 3rd instar midgut western corn rootworms. This screen allowed for the discovery of many novel sequences, many of which have extremely low or no homology to known sequences. This method provided the advantage of having no built in bias to genes that are frequently highly conserved across taxa. As a result, many novel targets for RNAi as well as known genes not previously shown to be sensitive to RNAi have been identified.

[0015] As such, methods and compositions are provided which employ one or more silencing elements that, when ingested by a pest, such as a Coleopteran plant pest or a *Diabrotica* plant pest, is capable of decreasing the expression of a target sequence in the pest. In specific embodiments, the decrease in expression of the target sequence controls the pest and thereby the methods and compositions are capable of limiting damage to a plant or plant part. The present invention provides target polynucleotides as set forth in SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, or active variants and fragments thereof, and complements thereof, including, for example, SEQ ID NOS: 1, 9, 37, 45, 49, 61, 65, 77, 101, 113, 137, 141, 145, 149, 153, 157, 169, 173, 181, 185, 189, 205, 217, 225,233, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708,

709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof. Silencing elements comprising sequences, complementary sequences, active fragments or variants of these target polynucleotides are provided which, when ingested by or when contacting the pest, decrease the expression of one or more of the target sequences and thereby controls the pest (i.e., has insecticidal activity).

[0016] As used herein, by "controlling a pest" or "controls a pest" is intended any affect on a pest that results in limiting the damage that the pest causes. Controlling a pest includes, but is not limited to, killing the pest, inhibiting development of the pest, altering fertility or growth of the pest in such a manner that the pest provides less damage to the plant, decreasing the number of offspring produced, producing less fit pests, producing pests more susceptible to predator attack, or deterring the pests from eating the plant.

[0017] Reducing the level of expression of the target polynucleotide or the polypeptide encoded thereby, in the pest results in the suppression, control, and/or killing the invading pest. Reducing the level of expression of the target sequence of the pest will reduce the pest damage by at least about 2% to at least about 6%, at least about 5% to about 50%, at least about 10% to about 60%, at least about 30% to about 70%, at least about 40% to about 80%, or at least about 50% to about 90% or greater. Hence, the methods of the invention can be utilized to control pests, particularly, Coleopteran plant pests or a *Diabrotica* plant pest.

[0018] Assays measuring the control of a pest are commonly known in the art, as are methods to record nodal injury score. See, for example, Oleson et al. (2005) J. Econ. Entomol. 98:1-8. See, for example, the examples below.

[0019] The invention is drawn to compositions and methods for protecting plants from a plant pest, such as Coleopteran plant pests or *Diabrotica* plant pests or inducing resistance in a plant to a plant pest, such as Coleopteran plant pests or *Diabrotica* plant pests. As used herein "Coleopteran plant pest" is used to refer to any member of the Coleoptera order. Other plant pests that may be targeted by the methods and compositions of the present invention include, but are not limited to Mexican Bean Beetle (*Epilachna varivestis*), and Colorado potato beetle (*Leptinotarsa decemlineata*),

[0020] As used herein, the term "*Diabrotica* plant pest" is used to refer to any member of the *Diabrotica* genus. Accordingly, the compositions and methods are also useful in protecting plants against any *Diabrotica* plant pest including, for example, *Diabrotica adelpha; Diabrotica ameca-ameca; Diabrotica balteata; Diabrotica barberi; Diabrotica biannularis; Diabrotica cristata; Diabrotica decempunctata; Diabrotica dissimilis; Diabrotica lemniscata; Diabrotica limitata* (including, for example, *Diabrotica limitata quindecimpuncata*); *Diabrotica longicornis; Diabrotica nummularis; Diabrotica porracea; Diabrotica scutellata; Diabrotica sexmaculata; Diabrotica speciosa* (including, for example, *Diabrotica speciosa speciosa*); *Diabrotica tibialis; Diabrotica undecimpunctata* (including, for example, Southern corn rootworm (*Diabrotica undecimpunctata*), *Diabrotica undecimpunctata duodecimnotata; Diabrotica undecimpunctata howardi* (spotted cucumber beetle); *Diabrotica undecimpunctata undecimpunctata* (western spotted cucumber beetle)); *Diabrotica virgifera* (including, for example, *Diabrotica virgifera virgifera* (western corn rootworm) and *Diabrotica virgifera zeae* (Mexican corn rootworm)); *Diabrotica viridula; Diabrotica wartensis; Diabrotica* sp. JJG335; *Diabrotica* sp. JJG336; *Diabrotica* sp. JJG341; *Diabrotica* sp. JJG356; *Diabrotica* sp. JJG362; and, *Diabrotica* sp. JJG365.

[0021] In specific embodiments, the *Diabrotica* plant pest comprises *D. virgifera virgifera, D. barberi, D. virgifera zeae, D. speciosa, D. speciosa* or *D. undecimpunctata* howardi.

II. Target Sequences

[0022] As used herein, a "target sequence" or "target polynucleotide" comprises any sequence in the pest that one desires to reduce the level of expression thereof. In specific embodiments, decreasing the level of the target sequence in the pest controls the pest. For instance, the target sequence may be essential for growth and development. While the target sequence can be expressed in any tissue of the pest, in specific embodiments, the sequences targeted for suppression in the pest are expressed in cells of the gut tissue of the pest, cells in the midgut of the pest, and cells lining the gut lumen or the midgut. Such target sequences can be involved in, for example, gut cell metabolism, growth or differentiation. Non-limiting examples of target sequences of the invention include a polynucleotide set forth in SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, including, for example, SEQ ID NOS: 1, 9, 37, 45, 49, 61, 65, 77, 101, 113, 137, 141, 145, 149, 153, 157, 169, 173, 181, 185, 189, 205, 217, 225,233, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586,

587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof. As exemplified elsewhere herein, decreasing the level of expression of one or more of these target sequences in a Coleopteran plant pest or a *Diabrotica* plant pest controls the pest.

III. Silencing Elements

[0023] By "silencing element" is intended a polynucleotide which when contacted by or ingested by a pest, is capable of reducing or eliminating the level or expression of a target polynucleotide or the polypeptide encoded thereby. The silencing element employed can reduce or eliminate the expression level of the target sequence by influencing the level of the target RNA transcript or, alternatively, by influencing translation and thereby affecting the level of the encoded polypeptide. Methods to assay for functional silencing elements that are capable of reducing or eliminating the level of a sequence of interest are disclosed elsewhere herein. A single polynucleotide employed in the methods of the invention can comprise one or more silencing elements to the same or different target polynucleotides. The silencing element can be produced in vivo (i.e., in a host cell such as a plant or microorganism) or in vitro.

[0024] In specific embodiments, the target sequence is not endogenous to the plant. In other embodiments, while the silencing element controls pests, preferably the silencing element has no effect on the normal plant or plant part.

[0025] As discussed in further detail below, silencing elements can include, but are not limited to, a sense suppression element, an antisense suppression element, a double stranded RNA, a siRNA, a amiRNA, a miRNA, or a hairpin suppression element. Silencing elements of the present invention may comprise a chimera where two or more sequences of the present invention or active fragments or variants, or complements thereof, are found in the same RNA molecule. Further, a sequence of the present invention or active fragment or variant, or complement thereof, may be present as more than one copy in a DNA construct, silencing element, DNA molecule or RNA molecule. In a hairpin or dsRNA molecule, the location of a sense or antisense sequence in the molecule, for example, in which sequence is transcribed first or is located on a particular terminus of the RNA molecule, is not limiting to the invention, and the invention is not to be limited by disclosures herein of a particular location for such a sequence. Non-limiting examples of silencing elements that can be employed to decrease expression of these target Coleopteran plant pest sequences or *Diabrotica* plant pest sequences comprise fragments and variants of the sense or antisense sequence or consists of the sense or antisense sequence of the sequence set forth in SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, including, for example, SEQ ID NOS: 1, 9, 37, 45, 49, 61, 65, 77, 101, 113, 137, 141, 145, 149, 153, 157, 169, 173, 181, 185, 189, 205, 217, 225,233, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof. The silencing element can further comprise additional sequences that advantageously effect transcription and/or the stability of a resulting transcript. For example, the silencing elements can comprise at least one thymine residue at the 3' end. This can aid in stabilization. Thus, the silencing elements can have at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more thymine residues at the 3' end. As discussed in further detail below, enhancer suppressor elements can also be employed in conjunction with the silencing elements disclosed herein.

[0026] By "reduces" or "reducing" the expression level of a polynucleotide or a polypeptide encoded thereby is intended to mean, the polynucleotide or polypeptide level of the target sequence is statistically lower than the polynucleotide level or polypeptide level of the same target sequence in an appropriate control pest which is not exposed to (i.e., has not ingested or come into contact with) the silencing element. In particular embodiments of the invention, reducing the polynucleotide level and/or the polypeptide level of the target sequence in a pest according to the invention results in less than 95%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, or less than 5% of the polynucleotide level, or the level of the polypeptide encoded thereby, of the same target sequence in an appropriate control pest. Methods to assay for the level of the RNA transcript, the level of the encoded polypeptide, or the activity of the polynucleotide or polypeptide are discussed elsewhere herein.

[0027] i. Sense Suppression Elements

[0028] As used herein, a "sense suppression element" comprises a polynucleotide designed to express an RNA molecule corresponding to at least a part of a target messenger RNA in the "sense" orientation. Expression of the RNA molecule comprising the sense suppression element reduces or eliminates the level of the target polynucleotide or the polypeptide encoded thereby. The polynucleotide comprising the sense suppression element may correspond to all or part of the sequence of the target polynucleotide, all or part of the 5' and/or 3' untranslated region of the target polynucleotide, all or part of the coding sequence of the target polynucleotide, or all or part of both the coding sequence and the untranslated regions of the target polynucleotide.

[0029] Typically, a sense suppression element has substantial sequence identity to the target polynucleotide, typically greater than about 65% sequence identity, greater than about 85% sequence identity, about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity. See, U.S. Pat. Nos. 5,283,184 and 5,034,323; herein incorporated by reference. The sense suppression element can be any length so long as it allows for the suppression of the targeted sequence. The sense suppression element can be, for example, 15, 16, 17, 18, 19, 20, 22, 25, 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 900, 1000, 1100, 1200, 1300 nucleotides or longer of the target polynucleotides set forth in any of SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, including, for example, SEQ ID NOS: 1, 9, 37, 45, 49, 61, 65, 77, 101, 113, 137, 141, 145, 149, 153, 157, 169, 173, 181, 185, 189, 205, 217, 225,233, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof. In other embodiments, the sense suppression element can be, for example, about 15-25, 19-35, 19-50, 25-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800 nucleotides or longer of the target polynucleotides set forth in any of SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469,

472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof.

[0030] ii. Antisense Suppression Elements

[0031] As used herein, an "antisense suppression element" comprises a polynucleotide which is designed to express an RNA molecule complementary to all or part of a target messenger RNA. Expression of the antisense RNA suppression element reduces or eliminates the level of the target polynucleotide. The polynucleotide for use in antisense suppression may correspond to all or part of the complement of the sequence encoding the target polynucleotide, all or part of the complement of the 5' and/or 3' untranslated region of the target polynucleotide, all or part of the complement of the coding sequence of the target polynucleotide, or all or part of the complement of both the coding sequence and the untranslated regions of the target polynucleotide. In addition, the antisense suppression element may be fully complementary (i.e., 100% identical to the complement of the target sequence) or partially complementary (i.e., less than 100% identical to the complement of the target sequence) to the target polynucleotide. In specific embodiments, the antisense suppression element comprises at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence complementarity to the target polynucleotide. Antisense suppression may be used to inhibit the expression of multiple proteins in the same plant. See, for example, U.S. Pat. No. 5,942,657. Furthermore, the antisense suppression element can be complementary to a portion of the target polynucleotide. Generally, sequences of at least 15, 16, 17, 18, 19, 20, 22, 25, 50, 100, 200, 300, 400, 450 nucleotides or greater of the sequence set forth in any of SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof may be used. Methods for using antisense suppression to inhibit the expression of endogenous genes in plants are described, for example, in Liu et al (2002) *Plant Physiol.* 129:1732-1743 and U.S. Pat. Nos. 5,759,829 and 5,942,657, each of which is herein incorporated by reference.

[0032] iii. Double Stranded RNA Suppression Element

[0033] A "double stranded RNA silencing element" or "dsRNA" comprises at least one transcript that is capable of

forming a dsRNA either before or after ingestion by a pest. Thus, a "dsRNA silencing element" includes a dsRNA, a transcript or polyribonucleotide capable of forming a dsRNA or more than one transcript or polyribonucleotide capable of forming a dsRNA. "Double stranded RNA" or "dsRNA" refers to a polyribonucleotide structure formed either by a single self-complementary RNA molecule or a polyribonucleotide structure formed by the expression of at least two distinct RNA strands. The dsRNA molecule(s) employed in the methods and compositions of the invention mediate the reduction of expression of a target sequence, for example, by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner. In the context of the present invention, the dsRNA is capable of reducing or eliminating the level or expression of a target polynucleotide or the polypeptide encoded thereby in a pest.

[0034] The dsRNA can reduce or eliminate the expression level of the target sequence by influencing the level of the target RNA transcript, by influencing translation and thereby affecting the level of the encoded polypeptide, or by influencing expression at the pre-transcriptional level (i.e., via the modulation of chromatin structure, methylation pattern, etc., to alter gene expression). See, for example, Verdel et al. (2004) *Science* 303:672-676; Pal-Bhadra et al. (2004) *Science* 303:669-672; Allshire (2002) *Science* 297:1818-1819; Volpe et al. (2002) *Science* 297:1833-1837; Jenuwein (2002) *Science* 297:2215-2218; and Hall et al. (2002) *Science* 297:2232-2237. Methods to assay for functional dsRNA that are capable of reducing or eliminating the level of a sequence of interest are disclosed elsewhere herein. Accordingly, as used herein, the term "dsRNA" is meant to encompass other terms used to describe nucleic acid molecules that are capable of mediating RNA interference or gene silencing, including, for example, short-interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), hairpin RNA, short hairpin RNA (shRNA), post-transcriptional gene silencing RNA (ptgsRNA), and others.

[0035] In specific embodiments, at least one strand of the duplex or double-stranded region of the dsRNA shares sufficient sequence identity or sequence complementarity to the target polynucleotide to allow for the dsRNA to reduce the level of expression of the target sequence. As used herein, the strand that is complementary to the target polynucleotide is the "antisense strand" and the strand homologous to the target polynucleotide is the "sense strand."

[0036] In another embodiment, the dsRNA comprises a hairpin RNA. A hairpin RNA comprises an RNA molecule that is capable of folding back onto itself to form a double stranded structure. Multiple structures can be employed as hairpin elements. In specific embodiments, the dsRNA suppression element comprises a hairpin element which comprises in the following order, a first segment, a second segment, and a third segment, where the first and the third segment share sufficient complementarity to allow the transcribed RNA to form a double-stranded stem-loop structure.

[0037] The "second segment" of the hairpin comprises a "loop" or a "loop region." These terms are used synonymously herein and are to be construed broadly to comprise any nucleotide sequence that confers enough flexibility to allow self-pairing to occur between complementary regions of a polynucleotide (i.e., segments **1** and **3** which form the stem of the hairpin). For example, in some embodiments, the loop region may be substantially single stranded and act as a spacer between the self-complementary regions of the hairpin stem-loop. In some embodiments, the loop region can comprise a random or nonsense nucleotide sequence and thus not share sequence identity to a target polynucleotide. In other embodiments, the loop region comprises a sense or an antisense RNA sequence or fragment thereof that shares identity to a target polynucleotide. See, for example, International Patent Publication No. WO 02/00904, herein incorporated by reference. In specific embodiments, the loop region can be optimized to be as short as possible while still providing enough intramolecular flexibility to allow the formation of the base-paired stem region. Accordingly, the loop sequence is generally less than 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 25, 20, 19, 18, 17, 16, 15, 10 nucleotides or less.

[0038] The "first" and the "third" segment of the hairpin RNA molecule comprise the base-paired stem of the hairpin structure. The first and the third segments are inverted repeats of one another and share sufficient complementarity to allow the formation of the base-paired stem region. In specific embodiments, the first and the third segments are fully complementary to one another. Alternatively, the first and the third segment may be partially complementary to each other so long as they are capable of hybridizing to one another to form a base-paired stem region. The amount of complementarity between the first and the third segment can be calculated as a percentage of the entire segment. Thus, the first and the third segment of the hairpin RNA generally share at least 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, up to and including 100% complementarity.

[0039] The first and the third segment are at least about 1000, 500, 475, 450, 425, 400, 375, 350, 325, 300, 250, 225, 200, 175, 150, 125, 100, 75, 60, 50, 40, 30, 25, 22, 20, 19, 18, 17, 16, 15 or 10 nucleotides in length. In specific embodiments, the length of the first and/or the third segment is about 10-100 nucleotides, about 10 to about 75 nucleotides, about 10 to about 50 nucleotides, about 10 to about 40 nucleotides, about 10 to about 35 nucleotides, about 10 to about 30 nucleotides, about 10 to about 25 nucleotides, about 10 to about 19 nucleotides, about 10 to about 20 nucleotides, about 19 to about 50 nucleotides, about 50 nucleotides to about 100 nucleotides, about 100 nucleotides to about 150 nucleotides, about 100 nucleotides to about 300 nucleotides, about 150 nucleotides to about 200 nucleotides, about 200 nucleotides to about 250 nucleotides, about 250 nucleotides to about 300 nucleotides, about 300 nucleotides to about 350 nucleotides, about 350 nucleotides to about 400 nucleotides, about 400 nucleotide to about 500 nucleotides, about 600 nt, about 700 nt, about 800 nt, about 900 nt, about 1000 nt, about 1100 nt, about 1200 nt, 1300 nt, 1400 nt, 1500 nt, 1600 nt, 1700 nt, 1800 nt, 1900 nt, 2000 nt or longer. In other embodiments, the length of the first and/or the third segment comprises at least 10-19 nucleotides, 10-20 nucleotides; 19-35 nucleotides, 20-35 nucleotides; 30-45 nucleotides; 40-50 nucleotides; 50-100 nucleotides; 100-300 nucleotides; about 500-700 nucleotides; about 700-900 nucleotides; about 900-1100 nucleotides; about 1300-1500 nucleotides; about 1500-1700 nucleotides; about 1700-1900 nucleotides; about 1900-2100 nucleotides; about 2100-2300 nucleotides; or about 2300-2500 nucleotides. See, for example, International Publication No. WO 0200904.

[0040] Hairpin molecules or double-stranded RNA molecules of the present invention may have more than one sequence of the present invention or active fragments or

variants, or complements thereof, found in the same portion of the RNA molecule. For example, in a chimeric hairpin structure, the first segment of a hairpin molecule comprises two polynucleotide sections, each with a different sequence of the present invention. For example, reading from one terminus of the hairpin, the first segment is composed of sequences from two separate genes (A followed by B). This first segment is followed by the second segment, the loop portion of the hairpin. The loop segment is followed by the third segment, where the complementary strands of the sequences in the first segment are found (B* followed by A*) in forming the stem-loop, hairpin structure, the stem contains SeqA-A* at the distal end of the stem and SeqB-B* proximal to the loop region.

[0041] In specific embodiments, the first and the third segment comprise at least 20 nucleotides having at least 85% complementary to the first segment. In still other embodiments, the first and the third segments which form the stem-loop structure of the hairpin comprises 3' or 5' overhang regions having unpaired nucleotide residues.

[0042] In specific embodiments, the sequences used in the first, the second, and/or the third segments comprise domains that are designed to have sufficient sequence identity to a target polynucleotide of interest and thereby have the ability to decrease the level of expression of the target polynucleotide. The specificity of the inhibitory RNA transcripts is therefore generally conferred by these domains of the silencing element. Thus, in some embodiments of the invention, the first, second and/or third segment of the silencing element comprise a domain having at least 10, at least 15, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 40, at least 50, at least 100, at least 200, at least 300, at least 500, at least 1000, or more than 1000 nucleotides that share sufficient sequence identity to the target polynucleotide to allow for a decrease in expression levels of the target polynucleotide when expressed in an appropriate cell. In other embodiments, the domain is between about 15 to 50 nucleotides, about 19-35 nucleotides, about 20-35 nucleotides, about 25-50 nucleotides, about 19 to 75 nucleotides, about 20 to 75 nucleotides, about 40-90 nucleotides about 15-100 nucleotides 10-100 nucleotides, about 10 to about 75 nucleotides, about 10 to about 50 nucleotides, about 10 to about 40 nucleotides, about 10 to about 35 nucleotides, about 10 to about 30 nucleotides, about 10 to about 25 nucleotides, about 10 to about 20 nucleotides, about 10 to about 19 nucleotides, about 50 nucleotides to about 100 nucleotides, about 100 nucleotides to about 150 nucleotides, about 150 nucleotides to about 200 nucleotides, about 200 nucleotides to about 250 nucleotides, about 250 nucleotides to about 300 nucleotides, about 300 nucleotides to about 350 nucleotides, about 350 nucleotides to about 400 nucleotides, about 400 nucleotide to about 500 nucleotides or longer. In other embodiments, the length of the first and/or the third segment comprises at least 10-20 nucleotides, at least 10-19 nucleotides, 20-35 nucleotides, 30-45 nucleotides, 40-50 nucleotides, 50-100 nucleotides, or about 100-300 nucleotides.

[0043] In specific embodiments, the domain of the first, the second, and/or the third segment has 100% sequence identity to the target polynucleotide. In other embodiments, the domain of the first, the second and/or the third segment having homology to the target polypeptide have at least 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater sequence identity to a region of the target polynucleotide. The sequence identity of the domains of the first, the second and/or the third segments to the target polynucleotide need only be sufficient to decrease expression of the target polynucleotide of interest. See, for example, Chuang and Meyerowitz (2000) *Proc. Natl. Acad. Sci. USA* 97:4985-4990; Stoutjesdijk et al. (2002) *Plant Physiol.* 129:1723-1731; Waterhouse and Helliwell (2003) *Nat. Rev. Genet.* 4:29-38; Pandolfini et al. *BMC Biotechnology* 3:7, and U.S. Patent Publication No. 20030175965; each of which is herein incorporated by reference. A transient assay for the efficiency of hpRNA constructs to silence gene expression in vivo has been described by Panstruga et al. (2003) *Mol. Biol. Rep.* 30:135-140, herein incorporated by reference.

[0044] The amount of complementarity shared between the first, second, and/or third segment and the target polynucleotide or the amount of complementarity shared between the first segment and the third segment (i.e., the stem of the hairpin structure) may vary depending on the organism in which gene expression is to be controlled. Some organisms or cell types may require exact pairing or 100% identity, while other organisms or cell types may tolerate some mismatching. In some cells, for example, a single nucleotide mismatch in the targeting sequence abrogates the ability to suppress gene expression. In these cells, the suppression cassettes of the invention can be used to target the suppression of mutant genes, for example, oncogenes whose transcripts comprise point mutations and therefore they can be specifically targeted using the methods and compositions of the invention without altering the expression of the remaining wild-type allele. In other organisms, holistic sequence variability may be tolerated as long as some 22 nt region of the sequence is represented in 100% homology between target polynucleotide and the suppression cassette.

[0045] Any region of the target polynucleotide can be used to design the domain of the silencing element that shares sufficient sequence identity to allow expression of the hairpin transcript to decrease the level of the target polynucleotide. For instance, the domain can be designed to share sequence identity to the 5' untranslated region of the target polynucleotide(s), the 3' untranslated region of the target polynucleotide(s), exonic regions of the target polynucleotide(s), intronic regions of the target polynucleotide(s), and any combination thereof. In specific embodiments, a domain of the silencing element shares sufficient homology to at least about 15, 16, 17, 18, 19, 20, 22, 25 or 30 consecutive nucleotides from about nucleotides 1-50, 25-75, 75-125, 50-100, 125-175, 175-225, 100-150, 150-200, 200-250, 225-275, 275-325, 250-300, 325-375, 375-425, 300-350, 350-400, 425-475, 400-450, 475-525, 450-500, 525-575, 575-625, 550-600, 625-675, 675-725, 600-650, 625-675, 675-725, 650-700, 725-825, 825-875, 750-800, 875-925, 925-975, 850-900, 925-975, 975-1025, 950-1000, 1000-1050, 1025-1075, 1075-1125, 1050-1100, 1125-1175, 1100-1200, 1175-1225, 1225-1275, 1200-1300, 1325-1375, 1375-1425, 1300-1400, 1425-1475, 1475-1525, 1400-1500, 1525-1575, 1575-1625, 1625-1675, 1675-1725, 1725-1775, 1775-1825, 1825-1875, 1875-1925, 1925-1975, 1975-2025, 2025-2075, 2075-2125, 2125-2175, 2175-2225, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 1900-2000 of the target sequence. In some instances to optimize the siRNA sequences employed in the hairpin, the synthetic oligodeoxyribonucleotide/RNAse H method can be used to deter-

mine sites on the target mRNA that are in a conformation that is susceptible to RNA silencing. See, for example, Vickers et al. (2003) *J. Biol. Chem* 278:7108-7118 and Yang et al. (2002) *Proc. Natl. Acad. Sci. USA* 99:9442-9447, herein incorporated by reference. These studies indicate that there is a significant correlation between the RNase-H-sensitive sites and sites that promote efficient siRNA-directed mRNA degradation.

[0046] The hairpin silencing element may also be designed such that the sense sequence or the antisense sequence do not correspond to a target polynucleotide. In this embodiment, the sense and antisense sequence flank a loop sequence that comprises a nucleotide sequence corresponding to all or part of the target polynucleotide. Thus, it is the loop region that determines the specificity of the RNA interference. See, for example, WO 02/00904, herein incorporated by reference.

[0047] In addition, transcriptional gene silencing (TGS) may be accomplished through use of a hairpin suppression element where the inverted repeat of the hairpin shares sequence identity with the promoter region of a target polynucleotide to be silenced. See, for example, Aufsatz et al. (2002) *PNAS* 99 (Suppl. 4):16499-16506 and Mette et al. (2000) *EMBO J* 19(19):5194-5201.

[0048] In other embodiments, the silencing element can comprise a small RNA (sRNA). sRNAs can comprise both micro RNA (miRNA) and short-interfering RNA (siRNA) (Meister and Tuschl (2004) *Nature* 431:343-349 and Bonetta et al. (2004) *Nature Methods* 1:79-86). miRNAs are regulatory agents comprising about 19 to about 24 ribonucleotides in length which are highly efficient at inhibiting the expression of target polynucleotides. See, for example Javier et al. (2003) *Nature* 425: 257-263, herein incorporated by reference. For miRNA interference, the silencing element can be designed to express a dsRNA molecule that forms a hairpin structure or partially base-paired structure containing 19, 20, 21, 22, 23, 24 or 25-nucleotide sequence that is complementary to the target polynucleotide of interest. The miRNA can be synthetically made, or transcribed as a longer RNA which is subsequently cleaved to produce the active miRNA. Specifically, the miRNA can comprise 19 nucleotides of the sequence having homology to a target polynucleotide in sense orientation and 19 nucleotides of a corresponding antisense sequence that is complementary to the sense sequence. The miRNA can be an "artificial miRNA" or "amiRNA" which comprises a miRNA sequence that is synthetically designed to silence a target sequence. When expressing an miRNA the final (mature) miRNA is present in a duplex in a precursor backbone structure, the two strands being referred to as the miRNA (the strand that will eventually basepair with the target) and miRNA*(star sequence). It has been demonstrated that miRNAs can be transgenically expressed and target genes of interest efficiently silenced (Highly specific gene silencing by artificial microRNAs in *Arabidopsis* Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D. Plant Cell. 2006 May; 18(5):1121-33. Epub 2006 Mar. 10 & Expression of artificial microRNAs in transgenic *Arabidopsis thaliana* confers virus resistance. Niu Q W, Lin S S, Reyes J L, Chen K C, Wu H W, Yeh S D, Chua N H. Nat Biotechnol. 2006 November; 24(11):1420-8. Epub 2006 Oct. 22. Erratum in: Nat Biotechnol. 2007 February; 25(2):254.)

[0049] The silencing element for miRNA interference comprises a miRNA primary sequence. The miRNA primary sequence comprises a DNA sequence having the miRNA and star sequences separated by a loop as well as additional sequences flanking this region that are important for processing. When expressed as an RNA, the structure of the primary miRNA is such as to allow for the formation of a hairpin RNA structure that can be processed into a mature miRNA. In some embodiments, the miRNA backbone comprises a genomic or cDNA miRNA precursor sequence, wherein said sequence comprises a native primary in which a heterologous (artificial) mature miRNA and star sequence are inserted.

[0050] As used herein, a "star sequence" is the sequence within a miRNA precursor backbone that is complementary to the miRNA and forms a duplex with the miRNA to form the stem structure of a hairpin RNA. In some embodiments, the star sequence can comprise less than 100% complementarity to the miRNA sequence. Alternatively, the star sequence can comprise at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% or lower sequence complementarity to the miRNA sequence as long as the star sequence has sufficient complementarity to the miRNA sequence to form a double stranded structure. In still further embodiments, the star sequence comprises a sequence having 1, 2, 3, 4, 5 or more mismatches with the miRNA sequence and still has sufficient complementarity to form a double stranded structure with the miRNA sequence resulting in production of miRNA and suppression of the target sequence.

[0051] The miRNA precursor backbones can be from any plant. In some embodiments, the miRNA precursor backbone is from a monocot. In other embodiments, the miRNA precursor backbone is from a dicot. In further embodiments, the backbone is from maize or soybean. MicroRNA precursor backbones have been described previously. For example, US20090155910A1 (WO 2009/079532) discloses the following soybean miRNA precursor backbones: 156c, 159, 166b, 168c, 396b and 398b, and US20090155909A1 (WO 2009/079548) discloses the following maize miRNA precursor backbones: 159c, 164h, 168a, 169r, and 396h. Each of these references is incorporated by reference in their entirety.

[0052] Thus, the primary miRNA can be altered to allow for efficient insertion of heterologous miRNA and star sequences within the miRNA precursor backbone. In such instances, the miRNA segment and the star segment of the miRNA precursor backbone are replaced with the heterologous miRNA and the heterologous star sequences, designed to target any sequence of interest, using a PCR technique and cloned into an expression construct. It is recognized that there could be alterations to the position at which the artificial miRNA and star sequences are inserted into the backbone. Detailed methods for inserting the miRNA and star sequence into the miRNA precursor backbone are described in, for example, US Patent Applications 20090155909A1 and US20090155910A1, herein incorporated by reference in their entirety.

[0053] When designing a miRNA sequence and star sequence, various design choices can be made. See, for example, Schwab R, et al. (2005) Dev Cell 8: 517-27. In non-limiting embodiments, the miRNA sequences disclosed herein can have a "U" at the 5'-end, a "C" or "G" at the 19th nucleotide position, and an "A" or "U" at the 10th nucleotide position. In other embodiments, the miRNA design is such that the miRNA have a high free delta-G as calculated using the ZipFold algorithm (Markham, N. R. & Zuker, M. (2005)

Nucleic Acids Res. 33: W577-W581.) Optionally, a one base pair change can be added within the 5' portion of the miRNA so that the sequence differs from the target sequence by one nucleotide.

[0054] The methods and compositions of the invention employ silencing elements that when transcribed "form" a dsRNA molecule. Accordingly, the heterologous polynucleotide being expressed need not form the dsRNA by itself, but can interact with other sequences in the plant cell or in the pest gut after ingestion to allow the formation of the dsRNA. For example, a chimeric polynucleotide that can selectively silence the target polynucleotide can be generated by expressing a chimeric construct comprising the target sequence for a miRNA or siRNA to a sequence corresponding to all or part of the gene or genes to be silenced. In this embodiment, the dsRNA is "formed" when the target for the miRNA or siRNA interacts with the miRNA present in the cell. The resulting dsRNA can then reduce the level of expression of the gene or genes to be silenced. See, for example, US Application Publication 2007-0130653, entitled "Methods and Compositions for Gene Silencing", herein incorporated by reference. The construct can be designed to have a target for an endogenous miRNA or alternatively, a target for a heterologous and/or synthetic miRNA can be employed in the construct. If a heterologous and/or synthetic miRNA is employed, it can be introduced into the cell on the same nucleotide construct as the chimeric polynucleotide or on a separate construct. As discussed elsewhere herein, any method can be used to introduce the construct comprising the heterologous miRNA.

e. Silencing Elements

[0055] A silencing element may comprise a chimeric construction molecule comprising two or more sequences of the present invention. For example, the chimeric construction may be a hairpin or dsRNA as disclosed herein. A chimera may comprise two or more sequences of the present invention. Providing at least two different sequences in a single silencing element may allow for targeting multiple genes using one silencing element and/or for example, one expression cassette. Targeting multiple genes may allow for slowing or reducing the possibility of resistance by the pest, and providing the multiple targeting ability in one expressed molecule may reduce the expression burden of the transformed plant or plant product, or provide topical treatments that are capable of targeting multiple hosts with one application.

IV. Variants and Fragments

[0056] By "fragment" is intended a portion of the polynucleotide or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a polynucleotide may encode protein fragments that retain the biological activity of the native protein. Alternatively, fragments of a polynucleotide that are useful as a silencing element do not need to encode fragment proteins that retain biological activity. Thus, fragments of a nucleotide sequence may range from at least about 10, about 15, about 16, about 17, about 18, about 19, nucleotides, about 20 nucleotides, about 22 nucleotides, about 50 nucleotides, about 75 nucleotides, about 100 nucleotides, 200 nucleotides, 300 nucleotides, 400 nucleotides, 500 nucleotides, 600 nucleotides, 700 nucleotides and up to the full-length polynucleotide employed in the invention. Alternatively, fragments of a nucleotide sequence may range from 1-50, 25-75, 75-125, 50-100, 125-175, 175-225, 100-150, 100-300, 150-200, 200-250, 225-275, 275-325, 250-300, 325-375, 375-425, 300-350, 350-400, 425-475, 400-450, 475-525, 450-500, 525-575, 575-625, 550-600, 625-675, 675-725, 600-650, 625-675, 675-725, 650-700, 725-825, 825-875, 750-800, 875-925, 925-975, 850-900, 925-975, 975-1025, 950-1000, 1000-1050, 1025-1075, 1075-1125, 1050-1100, 1125-1175, 1100-1200, 1175-1225, 1225-1275, 1200-1300, 1325-1375, 1375-1425, 1300-1400, 1425-1475, 1475-1525, 1400-1500, 1525-1575, 1575-1625, 1625-1675, 1675-1725, 1725-1775, 1775-1825, 1825-1875, 1875-1925, 1925-1975, 1975-2025, 2025-2075, 2075-2125, 2125-2175, 2175-2225, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 1900-2000 of any one of SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, including, for example, SEQ ID NOS: 1, 9, 37, 45, 49, 61, 65, 77, 101, 113, 137, 141, 145, 149, 153, 157, 169, 173, 181, 185, 189, 205, 217, 225,233, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ

ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof. Methods to assay for the activity of a desired silencing element are described elsewhere herein.

[0057] "Variants" is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a deletion and/or addition of one or more nucleotides at one or more internal sites within the native polynucleotide and/or a substitution of one or more nucleotides at one or more sites in the native polynucleotide. A variant of a polynucleotide that is useful as a silencing element will retain the ability to reduce expression of the target polynucleotide and, in some embodiments, thereby control a pest of interest. As used herein, a "native" polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively. For polynucleotides, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the polypeptides employed in the invention. Variant polynucleotides also include synthetically derived polynucleotide, such as those generated, for example, by using site-directed mutagenesis, but continue to retain the desired activity. Generally, variants of a particular polynucleotide of the invention (i.e., a silencing element) will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that particular polynucleotide as determined by sequence alignment programs and parameters described elsewhere herein.

[0058] Variants of a particular polynucleotide of the invention (i.e., the reference polynucleotide) can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant polynucleotide and the polypeptide encoded by the reference polynucleotide. Percent sequence identity between any two polypeptides can be calculated using sequence alignment programs and parameters described elsewhere herein. Where any given pair of polynucleotides employed in the invention is evaluated by comparison of the percent sequence identity shared by the two polypeptides they encode, the percent sequence identity between the two encoded polypeptides is at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity.

[0059] The following terms are used to describe the sequence relationships between two or more polynucleotides or polypeptides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", and, (d) "percentage of sequence identity."

[0060] (a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

[0061] (b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two polynucleotides. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

[0062] Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

[0063] (c) As used herein, "sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

[0064] (d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total

number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

[0065] A method is further provided for identifying a silencing element from the target polynucleotides set forth in SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, including, for example, SEQ ID NOS: 1, 9, 37, 45, 49, 61, 65, 77, 101, 113, 137, 141, 145, 149, 153, 157, 169, 173, 181, 185, 189, 205, 217, 225,233, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof. Such methods comprise obtaining a candidate fragment of any one of SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof, which is of sufficient length to act as a silencing element and thereby reduce the expression of the target polynucleotide and/or control a desired pest; expressing said candidate polynucleotide fragment in an appropriate expression cassette to produce a candidate silencing element and determining is said candidate polynucleotide fragment has the activity of a silencing element and thereby reduce the expression of the target polynucleotide and/or controls a desired pest. Methods of identifying such candidate fragments based on the desired pathway for suppression are known. For example, various bioinformatics programs can be employed to identify the region of the target polynucleotides that could be

exploited to generate a silencing element. See, for example, Elbahir et al. (2001) *Genes and Development* 15:188-200, Schwartz et al. (2003) *Cell* 115:199-208, Khvorova et al. (2003) *Cell* 115:209-216. See also, siRNA at Whitehead (jura.wi.mit.edu/bioc/siRNAext/) which calculates the binding energies for both sense and antisense siRNAs. See, also genscript.com/ssl-bin/app/rnai?op=known; Block-iT™ RNAi designer from Invitrogen and GenScript siRNA Construct Builder.

V. DNA Constructs

[0066] The use of the term "polynucleotide" is not intended to limit the present invention to polynucleotides comprising DNA. Those of ordinary skill in the art will recognize that polynucleotides can comprise ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules and synthetic analogues. The polynucleotides of the invention also encompass all forms of sequences including, but not limited to, single-stranded forms, double-stranded forms, hairpins, stem-and-loop structures, and the like.

[0067] The polynucleotide encoding the silencing element or in specific embodiments employed in the methods and compositions of the invention can be provided in expression cassettes for expression in a plant or organism of interest. It is recognized that multiple silencing elements including multiple identical silencing elements, multiple silencing elements targeting different regions of the target sequence, or multiple silencing elements from different target sequences can be used. In this embodiment, it is recognized that each silencing element can be contained in a single or separate cassette, DNA construct, or vector. As discussed, any means of providing the silencing element is contemplated. A plant or plant cell can be transformed with a single cassette comprising DNA encoding one or more silencing elements or separate cassettes comprising each silencing element can be used to transform a plant or plant cell or host cell. Likewise, a plant transformed with one component can be subsequently transformed with the second component. One or more silencing elements can also be brought together by sexual crossing. That is, a first plant comprising one component is crossed with a second plant comprising the second component. Progeny plants from the cross will comprise both components.

[0068] The expression cassette can include 5' and 3' regulatory sequences operably linked to the polynucleotide of the invention. "Operably linked" is intended to mean a functional linkage between two or more elements. For example, an operable linkage between a polynucleotide of the invention and a regulatory sequence (i.e., a promoter) is a functional link that allows for expression of the polynucleotide of the invention. Operably linked elements may be contiguous or non-contiguous. When used to refer to the joining of two protein coding regions, by operably linked is intended that the coding regions are in the same reading frame. The cassette may additionally contain at least one additional polynucleotide to be cotransformed into the organism. Alternatively, the additional polypeptide(s) can be provided on multiple expression cassettes. Expression cassettes can be provided with a plurality of restriction sites and/or recombination sites for insertion of the polynucleotide to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

[0069] The expression cassette can include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a polynucleotide comprising the silencing element employed in the methods and compositions of the invention, and a transcriptional and translational termination region (i.e., termination region) functional in plants. In other embodiment, the double stranded RNA is expressed from a suppression cassette. Such a cassette can comprise two convergent promoters that drive transcription of an operably linked silencing element. "Convergent promoters" refers to promoters that are oriented on either terminus of the operably linked silencing element such that each promoter drives transcription of the silencing element in opposite directions, yielding two transcripts. In such embodiments, the convergent promoters allow for the transcription of the sense and anti-sense strand and thus allow for the formation of a dsRNA.

[0070] The regulatory regions (i.e., promoters, transcriptional regulatory regions, and translational termination regions) and/or the polynucleotides employed in the invention may be native/analogous to the host cell or to each other. Alternatively, the regulatory regions and/or the polynucleotide employed in the invention may be heterologous to the host cell or to each other. As used herein, "heterologous" in reference to a sequence is a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous polynucleotide is from a species different from the species from which the polynucleotide was derived, or, if from the same/analogous species, one or both are substantially modified from their original form and/or genomic locus, or the promoter is not the native promoter for the operably linked polynucleotide. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence.

[0071] The termination region may be native with the transcriptional initiation region, may be native with the operably linked polynucleotide encoding the silencing element, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous) to the promoter, the polynucleotide comprising silencing element, the plant host, or any combination thereof. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau et al. (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon et al. (1991) *Genes Dev.* 5:141-149; Mogen et al. (1990) *Plant Cell* 2:1261-1272; Munroe et al. (1990) *Gene* 91:151-158; Ballas et al. (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi et al. (1987) *Nucleic Acids Res.* 15:9627-9639.

[0072] Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes

expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

[0073] In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, in vitro mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

[0074] A number of promoters can be used in the practice of the invention. The polynucleotide encoding the silencing element can be combined with constitutive, tissue-preferred, or other promoters for expression in plants.

[0075] Such constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Pat. No. 6,072,050; the core CaMV 35S promoter (Odell et al. (1985) *Nature* 313:810-812); rice actin (McElroy et al. (1990) *Plant Cell* 2:163-171); ubiquitin (Christensen et al. (1989) *Plant Mol. Biol.* 12:619-632 and Christensen et al. (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last et al. (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten et al. (1984) *EMBO J.* 3:2723-2730); ALS promoter (U.S. Pat. No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Pat. Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611.

[0076] An inducible promoter, for instance, a pathogen-inducible promoter could also be employed. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi et al. (1983) *Neth. J. Plant Pathol.* 89:245-254; Uknes et al. (1992) *Plant Cell* 4:645-656; and Van Loon (1985) *Plant Mol. Virol.* 4:111-116. See also WO 99/43819, herein incorporated by reference.

[0077] Additionally, as pathogens find entry into plants through wounds or insect damage, a wound-inducible promoter may be used in the constructions of the invention. Such wound-inducible promoters include potato proteinase inhibitor (pin II) gene (Ryan (1990) *Ann. Rev. Phytopath.* 28:425-449; Duan et al. (1996) *Nature Biotechnology* 14:494-498); wun1 and wun2, U.S. Pat. No. 5,428,148; win1 and win2 (Stanford et al. (1989) *Mol. Gen. Genet.* 215:200-208); systemin (McGurl et al. (1992) *Science* 225: 1570-1573); WIP1 (Rohmeier et al. (1993) *Plant Mol. Biol.* 22:783-792; Eckelkamp et al. (1993) *FEBS Letters* 323:73-76); MPI gene (Corderok et al. (1994) *Plant J.* 6(2):141-150); and the like, herein incorporated by reference.

[0078] Chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of the chemical induces gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression. Chemical-inducible promoters are known in the art and include, but are not limited to, the maize In2-2 promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco PR-la promoter, which is activated by salicylic acid. Other chemical-regulated promoters of interest include steroid-responsive promoters (see, for example, the glucocorticoid-inducible promoter in Schena et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:10421-10425 and McNellis et al. (1998) *Plant J.* 14(2):247-257) and tetracycline-inducible and tetracycline-repressible promoters (see, for example, Gatz et al. (1991) *Mol. Gen. Genet.* 227:229-237, and U.S. Pat. Nos. 5,814,618 and 5,789,156), herein incorporated by reference.

[0079] Tissue-preferred promoters can be utilized to target enhanced expression within a particular plant tissue. Tissue-preferred promoters include Yamamoto et al. (1997) *Plant J.* 12(2):255-265; Kawamata et al. (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen et al. (1997) *Mol. Gen Genet.* 254 (3):337-343; Russell et al. (1997) *Transgenic Res.* 6(2):157-168; Rinehart et al. (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp et al. (1996) *Plant Physiol.* 112(2):525-535; Canevascini et al. (1996) *Plant Physiol.* 112(2):513-524; Yamamoto et al. (1994) *Plant Cell Physiol.* 35(5):773-778; Lam (1994) *Results Probl. Cell Differ.* 20:181-196; Orozco et al. (1993) *Plant Mol Biol.* 23(6):1129-1138; Matsuoka et al. (1993) *Proc Natl. Acad. Sci. USA* 90(20):9586-9590; and Guevara-Garcia et al. (1993) *Plant J.* 4(3):495-505. Such promoters can be modified, if necessary, for weak expression.

[0080] Leaf-preferred promoters are known in the art. See, for example, Yamamoto et al. (1997) *Plant J.* 12(2):255-265; Kwon et al. (1994) *Plant Physiol.* 105:357-67; Yamamoto et al. (1994) *Plant Cell Physiol.* 35(5):773-778; Gotor et al. (1993) *Plant J.* 3:509-18; Orozco et al. (1993) *Plant Mol. Biol.* 23(6):1129-1138; and Matsuoka et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(20):9586-9590.

[0081] Root-preferred promoters are known and can be selected from the many available from the literature or isolated de novo from various compatible species. See, for example, Hire et al. (1992) *Plant Mol. Biol.* 20(2):207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner (1991) *Plant Cell* 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger et al. (1990) *Plant Mol. Biol.* 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); and Miao et al. (1991) *Plant Cell* 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also Bogusz et al. (1990) *Plant Cell* 2(7):633-641, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomentosa* are described. The promoters of these genes were linked to a β-glucuronidase reporter gene and introduced into both the nonlegume *Nicotiana tabacum* and the legume *Lotus corniculatus*, and in both instances root-specific promoter activity was preserved. Leach and Aoyagi (1991) describe their analysis of the promoters of the highly expressed rolC and rolD root-inducing genes of *Agrobacterium rhizogenes* (see Plant Science (Limerick) 79(1):69-76). They concluded that enhancer and tissue-preferred DNA determinants are dissociated in those promoters. Teeri et al. (1989) used gene fusion to lacZ to show that the *Agrobacterium* T-DNA gene encoding octopine synthase is especially active in the epi-

dermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in leaf tissue, an especially desirable combination of characteristics for use with an insecticidal or larvicidal gene (see *EMBO J.* 8(2): 343-350). The TR1' gene, fused to nptII (neomycin phosphotransferase II) showed similar characteristics. Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster et al. (1995) *Plant Mol. Biol.* 29(4):759-772); and rolB promoter (Capana et al. (1994) *Plant Mol. Biol.* 25(4):681-691. See also U.S. Pat. Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

[0082] In one embodiment of this invention the plant-expressed promoter is a vascular-specific promoter such as a phloem-specific promoter. A "vascular-specific" promoter, as used herein, is a promoter which is at least expressed in vascular cells, or a promoter which is preferentially expressed in vascular cells. Expression of a vascular-specific promoter need not be exclusively in vascular cells, expression in other cell types or tissues is possible. A "phloem-specific promoter" as used herein, is a plant-expressible promoter which is at least expressed in phloem cells, or a promoter which is preferentially expressed in phloem cells.

[0083] Expression of a phloem-specific promoter need not be exclusively in phloem cells, expression in other cell types or tissues, e.g., xylem tissue, is possible. In one embodiment of this invention, a phloem-specific promoter is a plant-expressible promoter at least expressed in phloem cells, wherein the expression in non-phloem cells is more limited (or absent) compared to the expression in phloem cells. Examples of suitable vascular-specific or phloem-specific promoters in accordance with this invention include but are not limited to the promoters selected from the group consisting of: the SCSV3, SCSV4, SCSV5, and SCSV7 promoters (Schunmann et al. (2003) *Plant Functional Biology* 30:453-60; the rolC gene promoter of *Agrobacterium rhizogenes*(Kiyokawa et al. (1994) *Plant Physiology* 104:801-02; Pandolfini et al. (2003) *BioMedCentral* (*BMC*) *Biotechnology* 3:7, (www.biomedcentral.com/1472-6750/3/7); Graham et al. (1997) *Plant Mol. Biol.* 33:729-35; Guivarc'h et al. (1996); Almon et al. (1997) *Plant Physiol.* 115:1599-607; the rolA gene promoter of *Agrobacterium rhizogenes* (Dehio et al. (1993) *Plant Mol. Biol.* 23:1199-210); the promoter of the *Agrobacterium tumefaciens* T-DNA gene 5 (Korber et al. (1991) *EMBO J.* 10:3983-91); the rice sucrose synthase RSs1 gene promoter (Shi et al. (1994) *J. Exp. Bot.* 45:623-31); the CoYMV or Commelina yellow mottle badnavirus promoter (Medberry et al. (1992) *Plant Cell* 4:185-92; Zhou et al. (1998) *Chin. J. Biotechnol.* 14:9-16); the CFDV or coconut foliar decay virus promoter (Rohde et al. (1994) *Plant Mol. Biol.* 27:623-28; Hehn and Rhode (1998) *J. Gen. Virol.* 79:1495-99); the RTBV or rice tungro bacilliform virus promoter (Yin and Beachy (1995) *Plant J.* 7:969-80; Yin et al. (1997) *Plant J* 12:1179-80); the pea glutamin synthase GS3A gene (Edwards et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:3459-63; Brears et al. (1991) *Plant J.* 1:235-44); the inv CD111 and inv CD141 promoters of the potato invertase genes (Hedley et al. (2000) *J. Exp. Botany* 51:817-21); the promoter isolated from *Arabidopsis* shown to have phloem-specific expression in tobacco by Kertbundit et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:5212-16); the VAHOX1 promoter region (Tornero et al. (1996) *Plant J.* 9:639-48); the pea cell wall invertase gene promoter (Zhang et al. (1996) *Plant Physiol.* 112:1111-17); the promoter of the endogenous cotton protein related to chitinase of US published patent application 20030106097, an acid invertase gene promoter from carrot (Ramloch-Lorenz et al. (1993) *The Plant J.* 4:545-54); the promoter of the sulfate transporter geneSultr1; 3 (Yoshimoto et al. (2003) *Plant Physiol.* 131:1511-17); a promoter of a sucrose synthase gene (Nolte and Koch (1993) *Plant Physiol.* 101:899-905); and the promoter of a tobacco sucrose transporter gene (Kuhn et al. (1997) Science 275-1298-1300).

[0084] Possible promoters also include the Black Cherry promoter for Prunasin Hydrolase (PH DL1.4 PRO) (U.S. Pat. No. 6,797,859), Thioredoxin H promoter from cucumber and rice (Fukuda A et al. (2005). *Plant Cell Physiol.* 46(11):1779-86), Rice (RSs1) (Shi, T. Wang et al. (1994). *J. Exp. Bot.* 45(274): 623-631) and maize sucrose synthese-1 promoters (Yang., N-S. et al. (1990) *PNAS* 87:4144-4148), PP2 promoter from pumpkin Guo, H. et al. (2004) *Transgenic Research* 13:559-566), At SUC2 promoter (Truernit, E. et al. (1995) *Planta* 196(3):564-70., At SAM-1 (S-adenosylmethionine synthetase) (Mijnsbrugge KV. et al. (1996) *Planr. Cell. Physiol.* 37(8): 1108-1115), and the Rice tungro bacilliform virus (RTBV) promoter (Bhattacharyya-Pakrasi et al. (1993)*Plant J.* 4(1):71-79).

[0085] The expression cassette can also comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). Additional selectable markers include phenotypic markers such as β-galactosidase and fluorescent proteins such as green fluorescent protein (GFP) (Su et al. (2004) *Biotechnol Bioeng* 85:610-9 and Fetter et al. (2004) *Plant Cell* 16:215-28), cyan florescent protein (CYP) (Bolte et al. (2004) *J. Cell Science* 117:943-54 and Kato et al. (2002) *Plant Physiol* 129:913-42), and yellow florescent protein (PhiYFP™ from Evrogen, see, Bolte et al. (2004) *J. Cell Science* 117:943-54). For additional selectable markers, see generally, Yarranton (1992) *Curr. Opin. Biotech.* 3:506-511; Christopherson et al. (1992) *Proc. Natl. Acad Sci. USA* 89:6314-6318; Yao et al. (1992) *Cell* 71:63-72; Reznikoff (1992) *Mol. Microbiol.* 6:2419-2422; Barkley et al. (1980) in *The Operon*, pp. 177-220; Hu et al. (1987) *Cell* 48:555-566; Brown et al. (1987) *Cell* 49:603-612; Figge et al. (1988) *Cell* 52:713-722; Deuschle et al. (1989) *Proc. Natl. Acad Sci. USA* 86:5400-5404; Fuerst et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2549-2553; Deuschle et al. (1990) *Science* 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines et al. (1993) *Proc. Natl. Acad Sci. USA* 90:1917-1921; Labow et al. (1990) *Mol. Cell. Biol.* 10:3343-3356; Zambretti et al. (1992) *Proc. Natl. Acad Sci. USA* 89:3952-3956; Baim et al. (1991) *Proc. Natl. Acad Sci. USA* 88:5072-5076; Wyborski et al. (1991) *Nucleic Acids Res.* 19:4647-4653; Hillenand-Wissman (1989) *Topics Mol. Struc. Biol.* 10:143-162; Degenkolb et al. (1991) *Antimicrob. Agents Chemother.* 35:1591-1595; Kleinschnidt et al. (1988) *Biochemistry* 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen et al. (1992) *Proc. Natl. Acad Sci. USA* 89:5547-5551; Oliva et al. (1992) *Antimicrob. Agents Chemother.* 36:913-919; Hlavka et al. (1985) *Handbook of Experimental Pharmacology*, Vol. 78

(Springer-Verlag, Berlin); Gill et al. (1988) *Nature* 334:721-724. Such disclosures are herein incorporated by reference. The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

VI. Compositions Comprising Silencing Elements

[0086] One or more of the polynucleotides comprising the silencing element can be provided as an external composition such as a spray or powder to the plant, plant part, seed, a pest, or an area of cultivation. In another example, a plant is transformed with a DNA construct or expression cassette for expression of at least one silencing element. In either composition, the silencing element, when ingested by an insect, can reduce the level of a target pest sequence and thereby control the pest (i.e., a Coleopteran plant pest including a *Diabrotica* plant pest, such as, *D. virgifera virgifera, D. barberi, D. virgifera zeae, D. speciosa*, or *D. undecimpunctata howardi*). It is recognized that the composition can comprise a cell (such as plant cell or a bacterial cell), in which a polynucleotide encoding the silencing element is stably incorporated into the genome and operably linked to promoters active in the cell. Compositions comprising a mixture of cells, some cells expressing at least one silencing element are also encompassed. In other embodiments, compositions comprising the silencing elements are not contained in a cell. In such embodiments, the composition can be applied to an area inhabited by a pest. In one embodiment, the composition is applied externally to a plant (i.e., by spraying a field or area of cultivation) to protect the plant from the pest. Methods of applying nucleotides in such a manner are known to those of skill in the art.

[0087] The composition of the invention can further be formulated as bait. In this embodiment, the compositions comprise a food substance or an attractant which enhances the attractiveness of the composition to the pest.

[0088] The composition comprising the silencing element can be formulated in an agriculturally suitable and/or environmentally acceptable carrier. Such carriers can be any material that the animal, plant or environment to be treated can tolerate. Furthermore, the carrier must be such that the composition remains effective at controlling a pest. Examples of such carriers include water, saline, Ringer's solution, dextrose or other sugar solutions, Hank's solution, and other aqueous physiologically balanced salt solutions, phosphate buffer, bicarbonate buffer and Tris buffer. In addition, the composition may include compounds that increase the half-life of a composition. Various insecticidal formulations can also be found in, for example, US Publications 2008/0275115, 2008/0242174, 2008/0027143, 2005/0042245, and 2004/0127520, each of which is herein incorporated by reference.

[0089] It is recognized that the polynucleotides comprising sequences encoding the silencing element can be used to transform organisms to provide for host organism production of these components, and subsequent application of the host organism to the environment of the target pest(s). Such host organisms include baculoviruses, bacteria, and the like. In this manner, the combination of polynucleotides encoding the silencing element may be introduced via a suitable vector into a microbial host, and said host applied to the environment, or to plants or animals.

[0090] The term "introduced" in the context of inserting a nucleic acid into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be stably incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

[0091] Microbial hosts that are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplana) of one or more crops of interest may be selected. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the sequences encoding the silencing element, and desirably, provide for improved protection of the components from environmental degradation and inactivation.

[0092] Such microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms such as bacteria, e.g., *Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylius, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc*, and *Alcaligenes*, fungi, particularly yeast, e.g., *Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae, Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacteria, Rhodopseudomonas spheroides, Xanthomonas campestris, Rhizobium melioti, Alcaligenes entrophus, Clavibacter xyli* and *Azotobacter vinlandir*, and phytosphere yeast species such as *Rhodotorula rubra, R. glutinis, R. marina, R. aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces rosues, S. odorus, Kluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

[0093] A number of ways are available for introducing the polynucleotide comprising the silencing element into the microbial host under conditions that allow for stable maintenance and expression of such nucleotide encoding sequences. For example, expression cassettes can be constructed which include the nucleotide constructs of interest operably linked with the transcriptional and translational regulatory signals for expression of the nucleotide constructs, and a nucleotide sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system that is functional in the host, whereby integration or stable maintenance will occur.

[0094] Transcriptional and translational regulatory signals include, but are not limited to, promoters, transcriptional initiation start sites, operators, activators, enhancers, other regulatory elements, ribosomal binding sites, an initiation codon, termination signals, and the like. See, for example, U.S. Pat. Nos. 5,039,523 and 4,853,331; EPO 0480762A2; Sambrook et al. (2000); *Molecular Cloning: A Laboratory Manual* (3rd ed.; Cold Spring Harbor Laboratory Press, Plainview, NY); Davis et al. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY); and the references cited therein.

[0095] Suitable host cells include the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and Gram-positive, include Enterobacteriaceae, such as *Escherichia, Erwinia, Shigella, Salmo-*

nella, and *Proteus; Bacillaceae; Rhizobiceae*, such as *Rhizobium; Spirillaceae*, such as photobacterium, *Zymomonas, Serratia, Aeromonas, Vibrio, Desulfovibrio, Spirillum; Lactobacillaceae; Pseudomonadaceae*, such as *Pseudomonas* and *Acetobacter; Azotobacteraceae* and *Nitrobacteraceae*. Among eukaryotes are fungi, such as *Phycomycetes* and *Ascomycetes*, which includes yeast, such as *Saccharomyces* and *Schizosaccharomyces*; and *Basidiomycetes* yeast, such as *Rhodotorula, Aureobasidium*, Sporobolomyces, and the like.

[0096] Characteristics of particular interest in selecting a host cell for purposes of the invention include ease of introducing the coding sequence into the host, availability of expression systems, efficiency of expression, stability in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

[0097] Host organisms of particular interest include yeast, such as *Rhodotorula* spp., *Aureobasidium* spp., *Saccharomyces* spp., and *Sporobolomyces* spp., phylloplane organisms such as *Pseudomonas* spp., *Erwinia* spp., and *Flavobacterium* spp., and other such organisms, including *Pseudomonas aeruginosa, Pseudomonas fluorescens, Saccharomyces cerevisiae, Bacillus thuringiensis, Escherichia coli, Bacillus subtilis*, and the like.

[0098] The sequences encoding the silencing elements encompassed by the invention can be introduced into microorganisms that multiply on plants (epiphytes) to deliver these components to potential target pests. Epiphytes, for example, can be gram-positive or gram-negative bacteria.

[0099] The silencing element can be fermented in a bacterial host and the resulting bacteria processed and used as a microbial spray in the same manner that *Bacillus thuringiensis* strains have been used as insecticidal sprays. Any suitable microorganism can be used for this purpose. By way of example, *Pseudomonas* has been used to express *Bacillus thuringiensis* endotoxins as encapsulated proteins and the resulting cells processed and sprayed as an insecticide Gaertner et al. (1993), in *Advanced Engineered Pesticides*, ed. L. Kim (Marcel Decker, Inc.).

[0100] Alternatively, the components of the invention are produced by introducing heterologous genes into a cellular host. Expression of the heterologous sequences results, directly or indirectly, in the intracellular production of the silencing element. These compositions may then be formulated in accordance with conventional techniques for application to the environment hosting a target pest, e.g., soil, water, and foliage of plants. See, for example, EPA 0192319, and the references cited therein.

[0101] In the present invention, a transformed microorganism can be formulated with an acceptable carrier into separate or combined compositions that are, for example, a suspension, a solution, an emulsion, a dusting powder, a dispersible granule, a wettable powder, and an emulsifiable concentrate, an aerosol, an impregnated granule, an adjuvant, a coatable paste, and also encapsulations in, for example, polymer substances.

[0102] Such compositions disclosed above may be obtained by the addition of a surface-active agent, an inert carrier, a preservative, a humectant, a feeding stimulant, an attractant, an encapsulating agent, a binder, an emulsifier, a dye, a UV protectant, a buffer, a flow agent or fertilizers, micronutrient donors, or other preparations that influence plant growth. One or more agrochemicals including, but not limited to, herbicides, insecticides, fungicides, bactericides, nematicides, molluscicides, acaracides, plant growth regulators, harvest aids, and fertilizers, can be combined with carriers, surfactants or adjuvants customarily employed in the art of formulation or other components to facilitate product handling and application for particular target pests. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g., natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders, or fertilizers. The active ingredients of the present invention (i.e., at least one silencing element) are normally applied in the form of compositions and can be applied to the crop area, plant, or seed to be treated. For example, the compositions may be applied to grain in preparation for or during storage in a grain bin or silo, etc. The compositions may be applied simultaneously or in succession with other compounds. Methods of applying an active ingredient or a composition that contains at least one silencing element include, but are not limited to, foliar application, seed coating, and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

[0103] Suitable surface-active agents include, but are not limited to, anionic compounds such as a carboxylate of, for example, a metal; carboxylate of a long chain fatty acid; an N-acylsarcosinate; mono- or di-esters of phosphoric acid with fatty alcohol ethoxylates or salts of such esters; fatty alcohol sulfates such as sodium dodecyl sulfate, sodium octadecyl sulfate, or sodium cetyl sulfate; ethoxylated fatty alcohol sulfates; ethoxylated alkylphenol sulfates; lignin sulfonates; petroleum sulfonates; alkyl aryl sulfonates such as alkyl-benzene sulfonates or lower alkylnaphtalene sulfonates, e.g., butyl-naphthalene sulfonate; salts of sulfonated naphthalene-formaldehyde condensates; salts of sulfonated phenol-formaldehyde condensates; more complex sulfonates such as the amide sulfonates, e.g., the sulfonated condensation product of oleic acid and N-methyl taurine; or the dialkyl sulfosuccinates, e.g., the sodium sulfonate or dioctyl succinate. Non-ionic agents include condensation products of fatty acid esters, fatty alcohols, fatty acid amides or fatty-alkyl- or alkenyl-substituted phenols with ethylene oxide, fatty esters of polyhydric alcohol ethers, e.g., sorbitan fatty acid esters, condensation products of such esters with ethylene oxide, e.g., polyoxyethylene sorbitan fatty acid esters, block copolymers of ethylene oxide and propylene oxide, acetylenic glycols such as 2,4,7,9-tetraethyl-5-decyn-4,7-diol, or ethoxylated acetylenic glycols. Examples of a cationic surface-active agent include, for instance, an aliphatic mono-, di-, or polyamine such as an acetate, naphthenate or oleate; or oxygen-containing amine such as an amine oxide of polyoxyethylene alkylamine; an amide-linked amine prepared by the condensation of a carboxylic acid with a di- or polyamine; or a quaternary ammonium salt.

[0104] Examples of inert materials include, but are not limited to, inorganic minerals such as kaolin, phyllosilicates,

carbonates, sulfates, phosphates, or botanical materials such as cork, powdered corncobs, peanut hulls, rice hulls, and walnut shells.

[0105] The compositions comprising the silencing element can be in a suitable form for direct application or as a concentrate of primary composition that requires dilution with a suitable quantity of water or other dilutant before application.

[0106] The compositions (including the transformed microorganisms) can be applied to the environment of an insect pest (such as a Coleoptera plant pest or a *Diabrotica* plant pest) by, for example, spraying, atomizing, dusting, scattering, coating or pouring, introducing into or on the soil, introducing into irrigation water, by seed treatment or general application or dusting at the time when the pest has begun to appear or before the appearance of pests as a protective measure. For example, the composition(s) and/or transformed microorganism(s) may be mixed with grain to protect the grain during storage. It is generally important to obtain good control of pests in the early stages of plant growth, as this is the time when the plant can be most severely damaged. The compositions can conveniently contain another insecticide if this is thought necessary. In an embodiment of the invention, the composition(s) is applied directly to the soil, at a time of planting, in granular form of a composition of a carrier and dead cells of a *Bacillus* strain or transformed microorganism of the invention. Another embodiment is a granular form of a composition comprising an agrochemical such as, for example, a herbicide, an insecticide, a fertilizer, in an inert carrier, and dead cells of a *Bacillus* strain or transformed microorganism of the invention.

VII. Plants, Plant Parts, and Methods of Introducing Sequences into Plants

[0107] In one embodiment, the methods of the invention involve introducing a polynucleotide into a plant. "Introducing" is intended to mean presenting to the plant the polynucleotide in such a manner that the sequence gains access to the interior of a cell of the plant. The methods of the invention do not depend on a particular method for introducing a sequence into a plant, only that the polynucleotide or polypeptides gains access to the interior of at least one cell of the plant. Methods for introducing polynucleotides into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

[0108] "Stable transformation" is intended to mean that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof. "Transient transformation" is intended to mean that a polynucleotide is introduced into the plant and does not integrate into the genome of the plant or a polypeptide is introduced into a plant.

[0109] Transformation protocols as well as protocols for introducing polypeptides or polynucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing polypeptides and polynucleotides into plant cells include microinjection (Crossway et al. (1986) *Biotechniques* 4:320-334), electroporation (Riggs et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606, *Agrobacterium*-mediated transformation (U.S. Pat. Nos. 5,563,055 and 5,981,840), direct gene transfer (Paszkowski et al. (1984) *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, U.S. Pat. Nos. 4,945,050; 5,879,918; 5,886,244; and, 5,932,782; Tomes et al. (1995) in Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe et al. (1988) *Biotechnology* 6:923-926); and Lec transformation (WO 00/28058). Also see Weissinger et al. (1988) *Ann. Rev. Genet.* 22:421-477; Sanford et al. (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou et al. (1988) *Plant Physiol.* 87:671-674 (soybean); McCabe et al. (1988) *Bio Technology* 6:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P: 175-182 (soybean); Singh et al. (1998) *Theor. Appl. Genet.* 96:319-324 (soybean); Datta et al. (1990) *Biotechnology* 8:736-740 (rice); Klein et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein et al. (1988) *Biotechnology* 6:559-563 (maize); U.S. Pat. Nos. 5,240,855; 5,322,783; and, 5,324,646; Klein et al. (1988) *Plant Physiol.* 91:440-444 (maize); Fromm et al. (1990) *Biotechnology* 8:833-839 (maize); Hooykaas-Van Slogteren et al. (1984) *Nature* (London) 311:763-764; U.S. Pat. No. 5,736,369 (cereals); Bytebier et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet et al. (1985) in *The Experimental Manipulation of Ovule Tissues*, ed. Chapman et al. (Longman, New York), pp. 197-209 (pollen); Kaeppler et al. (1990) *Plant Cell Reports* 9:415-418 and Kaeppler et al. (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin et al. (1992) *Plant Cell* 4:1495-1505 (electroporation); Li et al. (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford (1995) *Annals of Botany* 75:407-413 (rice); Osjoda et al. (1996) *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

[0110] In specific embodiments, the silencing element sequences of the invention can be provided to a plant using a variety of transient transformation methods. Such transient transformation methods include, but are not limited to, the introduction of the protein or variants and fragments thereof directly into the plant or the introduction of the transcript into the plant. Such methods include, for example, microinjection or particle bombardment. See, for example, Crossway et al. (1986) Mol Gen. Genet. 202:179-185; Nomura et al. (1986) Plant Sci. 44:53-58; Hepler et al. (1994) Proc. Natl. Acad. Sci. 91: 2176-2180 and Hush et al. (1994) The Journal of Cell Science 107:775-784, all of which are herein incorporated by reference. Alternatively, polynucleotides can be transiently transformed into the plant using techniques known in the art. Such techniques include viral vector systems and the precipitation of the polynucleotide in a manner that precludes subsequent release of the DNA. Thus, the transcription from the particle-bound DNA can occur, but the frequency with which it is released to become integrated into the genome is greatly reduced. Such methods include the use of particles coated with polyethylimine (PEI; Sigma #P3143).

[0111] In other embodiments, the polynucleotide of the invention may be introduced into plants by contacting plants with a virus or viral nucleic acids. Generally, such methods involve incorporating a nucleotide construct of the invention within a viral DNA or RNA molecule. Further, it is recognized that promoters of the invention also encompass promoters utilized for transcription by viral RNA polymerases. Methods for introducing polynucleotides into plants and expressing a protein encoded therein, involving viral DNA or RNA molecules, are known in the art. See, for example,

U.S. Pat. Nos. 5,889,191, 5,889,190, 5,866,785, 5,589,367, 5,316,931, and Porta et al. (1996) *Molecular Biotechnology* 5:209-221; herein incorporated by reference.

[0112] Methods are known in the art for the targeted insertion of a polynucleotide at a specific location in the plant genome. In one embodiment, the insertion of the polynucleotide at a desired genomic location is achieved using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853, all of which are herein incorporated by reference. Briefly, the polynucleotide of the invention can be contained in transfer cassette flanked by two non-recombinogenic recombination sites. The transfer cassette is introduced into a plant having stably incorporated into its genome a target site which is flanked by two non-recombinogenic recombination sites that correspond to the sites of the transfer cassette. An appropriate recombinase is provided and the transfer cassette is integrated at the target site. The polynucleotide of interest is thereby integrated at a specific chromosomal position in the plant genome.

[0113] The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick et al. (1986) *Plant Cell Reports* 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting progeny having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved. In this manner, the present invention provides transformed seed (also referred to as "transgenic seed") having a polynucleotide of the invention, for example, an expression cassette of the invention, stably incorporated into their genome.

[0114] As used herein, the term plant includes plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers, and the like. Grain is intended to mean the mature seed produced by commercial growers for purposes other than growing or reproducing the species. Progeny, variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced polynucleotides.

[0115] The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plant species of interest include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (e.g., *B. napus, B. rapa, B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor, Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense, Gossypium hirsutum*), sweet potato (*Ipomoea batatus*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), *papaya* (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables, ornamentals, and conifers.

[0116] Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (e.g., *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and *chrysanthemum.*

[0117] Conifers that may be employed in practicing the present invention include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), *ponderosa* pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*). In specific embodiments, plants of the present invention are crop plants (for example, corn, alfalfa, sunflower, *Brassica*, soybean, cotton, safflower, peanut, sorghum, wheat, millet, tobacco, etc.). In other embodiments, corn and soybean plants and sugarcane plants are optimal, and in yet other embodiments corn plants are optimal.

[0118] Other plants of interest include grain plants that provide seeds of interest, oil-seed plants, and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Oil-seed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

VIII. Methods of Use

[0119] Methods of the invention comprise methods for controlling a pest (i.e., a Coleopteran plant pest, including a *Diabrotica* plant pest, such as, *D. virgifera virgifera, D. barberi, D. virgifera zeae, D. speciosa*, or *D. undecimpunctata howardi*). The method comprises feeding or applying to a pest a composition comprising a silencing element of the invention, wherein said silencing element, when ingested or contacted by a pest (i.e., a Coleopteran plant pest including a *Diabrotica* plant pest, such as, *D. virgifera virgifera, D. barberi, D. virgifera zeae, D. speciosa*, or *D. undecimpunctata howardi*), reduces the level of a target polynucleotide of the pest and thereby controls the pest. The pest can be fed the silencing element in a variety of ways. For example, in one embodiment, the polynucleotide comprising the silencing element is introduced into a plant.

[0120] As the Coleopteran plant pest or *Diabrotica* plant pest feeds on the plant or part thereof expressing these

sequences, the silencing element is delivered to the pest. When the silencing element is delivered to the plant in this manner, it is recognized that the silencing element can be expressed constitutively or alternatively, it may be produced in a stage-specific manner by employing the various inducible or tissue-preferred or developmentally regulated promoters that are discussed elsewhere herein. In specific embodiments, the silencing element is expressed in the roots, stalk or stem, leaf including pedicel, xylem and phloem, fruit or reproductive tissue, silk, flowers and all parts therein or any combination thereof.

[0121] In another method, a composition comprising at least one silencing element of the invention is applied to a plant. In such embodiments, the silencing element can be formulated in an agronomically suitable and/or environmentally acceptable carrier, which is preferably, suitable for dispersal in fields. In addition, the carrier can also include compounds that increase the half life of the composition. In specific embodiments, the composition comprising the silencing element is formulated in such a manner such that it persists in the environment for a length of time sufficient to allow it to be delivered to a pest. In such embodiments, the composition can be applied to an area inhabited by a pest. In one embodiment, the composition is applied externally to a plant (i.e., by spraying a field) to protect the plant from pests.

[0122] In certain embodiments, the constructs of the present invention can be stacked with any combination of polynucleotide sequences of interest in order to create plants with a desired trait. A trait, as used herein, refers to the phenotype derived from a particular sequence or groups of sequences. For example, the polynucleotides of the present invention may be stacked with any other polynucleotides encoding polypeptides having pesticidal and/or insecticidal activity, such as other *Bacillus thuringiensis* toxic proteins (described in U.S. Pat. Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; and Geiser et al. (1986) *Gene* 48:109), lectins (Van Damme et al. (1994) *Plant Mol. Biol.* 24:825, pentin (described in U.S. Pat. No. 5,981,722), and the like. The combinations generated can also include multiple copies of any one of the polynucleotides of interest. The polynucleotides of the present invention can also be stacked with any other gene or combination of genes to produce plants with a variety of desired trait combinations including, but not limited to, traits desirable for animal feed such as high oil genes (e.g., U.S. Pat. No. 6,232,529); balanced amino acids (e.g., hordothionins (U.S. Pat. Nos. 5,990,389; 5,885,801; 5,885,802; and 5,703,409); barley high lysine (Williamson et al. (1987) *Eur. J. Biochem.* 165:99-106; and WO 98/20122) and high methionine proteins (Pedersen et al. (1986) *J. Biol. Chem.* 261:6279; Kirihara et al. (1988) *Gene* 71:359; and Musumura et al. (1989) *Plant Mol. Biol.* 12:123)); increased digestibility (e.g., modified storage proteins (U.S. application Ser. No. 10/053,410, filed Nov. 7, 2001); and thioredoxins (U.S. application Ser. No. 10/005,429, filed Dec. 3, 2001)); the disclosures of which are herein incorporated by reference.

[0123] The polynucleotides of the present invention can also be stacked with traits desirable for disease or herbicide resistance (e.g., fumonisin detoxification genes (U.S. Pat. No. 5,792,931); avirulence and disease resistance genes (Jones et al. (1994) *Science* 266:789; Martin et al. (1993) *Science* 262:1432; Mindrinos et al. (1994) *Cell* 78:1089); acetolactate synthase (ALS) mutants that lead to herbicide resistance such as the S4 and/or Hra mutations; inhibitors of glutamine synthase such as phosphinothricin or basta (e.g., bar gene); and glyphosate resistance (EPSPS gene)); and traits desirable for processing or process products such as high oil (e.g., U.S. Pat. No. 6,232,529); modified oils (e.g., fatty acid desaturase genes (U.S. Pat. No. 5,952,544; WO 94/11516)); modified starches (e.g., ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE), and starch debranching enzymes (SDBE)); and polymers or bioplastics (e.g., U.S. Pat. No. 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase, and acetoacetyl-CoA reductase (Schubert et al. (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)); the disclosures of which are herein incorporated by reference. One could also combine the polynucleotides of the present invention with polynucleotides providing agronomic traits such as male sterility (e.g., see U.S. Pat. No. 5,583,210), stalk strength, drought resistance (e.g., U.S. Pat. No. 7,786,353), flowering time, or transformation technology traits such as cell cycle regulation or gene targeting (e.g., WO 99/61619, WO 00/17364, and WO 99/25821); the disclosures of which are herein incorporated by reference.

[0124] These stacked combinations can be created by any method including, but not limited to, cross-breeding plants by any conventional or TopCross methodology, or genetic transformation. If the sequences are stacked by genetically transforming the plants (i.e., molecular stacks), the polynucleotide sequences of interest can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of the polynucleotide of interest. This may be combined with any combination of other suppression cassettes or overexpression cassettes to generate the desired combination of traits in the plant. It is further recognized that polynucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853, all of which are herein incorporated by reference.

[0125] The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1: In Vitro Transcript dsRNA Screening Method

[0126] A cDNA library was produced from neonate western corn rootworm larvae by standard methods. A selected cDNA clone containing an expressed sequence tag was amplified in a PCR using universal primers to the plasmid backbone and flanking the EST insert. The universal primers also contained T7 RNA polymerase sites. Product of the PCR reaction was used as the template for an in vitro

transcription (IVT) reaction to produce long double stranded RNAs. Following enzymatic digestion and removal of the DNA template and single stranded RNA, the IVT reaction products were incorporated into artificial insect diet as described below.

[0127] Different target selection strategies were used in this invention to identify RNAi active targets with insecticidal activities in corn rootworn diet based assay. cDNA libraries were produced from neonate or midgut of 3rd instar western corn rootworm larvae by standard methods. Randomly selected cDNA clones containing an expressed sequence tag (EST) were amplified in a PCR using target specific primers (forward and reverse Table 1), and provided in the sequence listing included herein, to generate DNA templates. The target specific primers also contain T7 RNA polymerase sites (T7 sequence at 5' end of each primer). Second set of cDNA clones was selected based on homology to known lethal genes from other insects, primarily *Drosophila melanogaster*. A third set of genes was tested based on involvement in proteosome functions. Identification of these genes was based on a progressive homology search beginning with a list of proteosome genes identified in humans cross referenced to the Tribolium genome database. Hits from Tribolium were then used to parse western corn rootworm sequence database. Proteosome genes were categorized as 26S subunit non ATPase, 26S subunit ATPase, alpha type, and beta type genes.

[0128] Region(s) of WCRW genes were produced by PCR followed by in vitro transcription (IVT) to produce long double stranded RNAs. The IVT reaction products are quantified in gel and incorporated into artificial insect diet for first-round IVT screening (FIS) as described below.

Insect Bioassays

[0129] dsRNAs were incorporated into standard WCRW artificial diet at a final concentration of 50 ppm in a 96 well microtiter plate format. 5 μl of the IVT reaction (300 ng/ul) were added to a given well of a 96 well microtiter plate. 25 μl of molten lowmelt Western corn rootworm diet were added to the sample and shaken on an orbital shaker to mix the sample and diet. Once the diet has solidified, eight wells were used for each RNA sample. Preconditioned 1st instar WCRW (neonate insects were placed on neutral diet for 24 hours prior to transfer to test material) were added to the 96 well microtiter plates at a rate of 3-5 insects/well. Plates were sealed with mylar which was then punctured twice above each well of the microtiter plate using a superfine insect collection pin. To prevent drying of the diet, plates were first placed inside a plastic bag with a slightly damp cloth and the bags were placed inside an incubator set at 28 C and 70 RH. The assay was scored for mortality and stunting affects after 7 days and an average was determined based on assignment of numeric values to each category of impact (3=mortality, 2=severe stunting, 1=stunting, 0=no affect). The number reported in this and all diet assay tables reflect the average score across all observations. A score of 3 represents complete mortality across all observations. A score of 2.5 would indicate half the wells demonstrating mortality and half scored as severe stunting. The assay results can be found in Table 1A.

[0130] DNA sequences which encode double stranded RNAs which were shown to have insecticidal activity (average score above 1.5) against corn rootworms using the assay described in Example 1 are listed in Table 1. To identify full length of cDNA or full open-reading frame of RNAi active target gene, full insert sequencing for EST clones and transcriptome analyses of midgut RNA samples were conducted. Sequences of all target transcripts containing full length cDNA or longer transcripts were also listed in Table 1. Some of these sequences were used for RNAi active fragment search.

Example 2. Sequences Having Insecticidal Activity

[0131] DNA sequences which encode double stranded RNAs which were shown to have insecticidal activity against corn rootworms using the assay described in Example 1 are set forth below. Non-limiting examples of target polynucleotides are set forth below in Table 1A and B, and SEQ ID NOS: 1, 4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, including, for example, SEQ ID NOS: 1, 9, 37, 45, 49, 61, 65, 77, 101, 113, 137, 141, 145, 149, 153, 157, 169, 173, 181, 185, 189, 205, 217, 225,233, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690,

691, 692, and active variants and fragments thereof, and complements thereof, and SEQ ID NOS: 4, 140, 144, 148, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721 and active variants and fragments thereof, and complements thereof.

[0132] Subregions of efficacious dsRNAs were designed to improve insecticidal activities in diet and dsRNA expression in planta. These fragments were assayed in the same manner as the original FIS assays described above. Regions demonstrating a severe impact on larval phenotype (mortality or severe growth retardation) were advanced to informal inhibitory concentration (IC50) assays. IC50 assays used doses starting at 50 ppm and progressing downward by ½ step dilutions through 25, 12.5, 6, 3, 1.5, and 0.75 pppm. 12 observations were included for each rate.

[0133] Assay methods were the same as described above for primary screens. Calculations of inhibition relied on scoring for both mortality and severe stunting Selected fragments were advanced to formal dose response assays where both LC50 and IC50 values were calculated and described in Table 2 (Seq No. 561 to 692). These assays included an initial range finding assay followed by dose response assays for selected ranges including 3 replications of the experiment. Fragments with confirmed IC50 values below 2 ppm were advanced to plant transformation vector construction.

[0134] The proteosome alpha subunit type 3 (PAT3) target gene was used as a model for gene and construct optimization. As a first step, the gene was divided into ⅓, ⅙, and 1/12 size fragments (f). In addition, f11-13 represent spanning segments over the boundaries of the first four ⅙th fragments. FIG. **5** provides a diagram of the fragments of PAT3.

[0135] Plant preferred fragments were identified from active RNAi gene targets and tested in dsRNA artificial diet assays. Selection of these plant preferred regions was based on avoiding destabilizing elements and motifs, or regions with unsuitable base composition. Homology assessments were also employed to avoid potential non target organisms. Finally, fragments with a size range of 150-250 bp were preferred. All rules were considered in selecting fragments but fragments were not excluded from consideration based on any one rule. The table 2 includes data for initial FIS samples and subsequent fragments insecticidal assay. Selected samples were advanced to IC50 and LC50 determinations.

Example 3. Identify RNAi Active Targets from Other Insects

[0136] To identify RNAi active genes from other important corn pests or no-target insects, transcriptome experiments were completed using 3rd instar larvae from Northern corn rootworm (*Diabrotica barberi*), Southern corn rootworm (*Diabrotica undecimpunctata*), Mexican Bean Beetle (*Epilachna varivestis*), Colorado potato beetle (*Leptinotarsa decemlineata*), Insidious flower bug (*Orius insidiosus*) and Spotted Lady Beetle (*Coleomegilla maculata*, [CMAC]).

[0137] Homologous transcripts of RNAi active leads were listed in Table 3 (Seq No. 693 to 723). This sequence data is important for designing fragments to suppress target pest genes and avoid knockdown same gene in no target insects.

Example 4. Insecticidal RNA Targets in WCRW Midgut

[0138] Two RNAi active targets Ryanr and HP2 (Table 1 and Table 2) were identified through random cDNA FIS screening. Ryanr was identified in a previous FIS screening (US 2011/0054007A1). Fragments of these targets showed very strong insecticidal activities. Homologous searches reveal that Ryanr and HP2 showed 54% and 49% identity to *Drosophila* Ssk and Mesh, respectively. The Mesh-Ssk protein complex is required for septate junction formation in the *Drosophila* midgut. See the amino acid sequence alignment of WCRW Ryanr and *Drosophila* Ssk in FIG. **4**.

Example 5. Transformation of Maize

[0139] Immature maize embryos from greenhouse donor plants are bombarded with a plasmid containing the silencing element of the invention operably linked to either a tissue specific, tissue selective, or constitutive promoter and the selectable marker gene PAT (Wohlleben et al. (1988) *Gene* 70:25-37), which confers resistance to the herbicide Bialaphos. In one embodiment, the constructs will express a long double stranded RNA of the target sequence set forth in table 1. Such a construct can be linked to the dMMB promoter. Alternatively, the selectable marker gene is provided on a separate plasmid. Transformation is performed as follows. Media recipes follow below.

Preparation of Target Tissue

[0140] The ears are husked and surface sterilized in 30% Clorox bleach plus 0.5% Micro detergent for 20 minutes, and rinsed two times with sterile water. The immature embryos are excised and placed embryo axis side down (scutellum side up), 25 embryos per plate, on 560Y medium for 4 hours and then aligned within the 2.5 cm target zone in preparation for bombardment.

[0141] A plasmid vector comprising the silencing element of interest operably linked to either the tissue specific, tissue selective, or constitutive promoter is made. This plasmid DNA plus plasmid DNA containing a PAT selectable marker is precipitated onto 1.1 μm (average diameter) tungsten pellets using a $CaCl_2$) precipitation procedure as follows: 100 μl prepared tungsten particles in water; 10 μl (1 μg) DNA in Tris EDTA buffer (1 μg total DNA); 100 μl 2.5 M $CaCl_2$; and, 10 μl 0.1 M spermidine.

[0142] Each reagent is added sequentially to the tungsten particle suspension, while maintained on the multitube vortexer. The final mixture is sonicated briefly and allowed to incubate under constant vortexing for 10 minutes. After the precipitation period, the tubes are centrifuged briefly, liquid removed, washed with 500 ml 100% ethanol, and centrifuged for 30 seconds. Again the liquid is removed, and 105 μl 100% ethanol is added to the final tungsten particle pellet. For particle gun bombardment, the tungsten/DNA particles are briefly sonicated and 10 μl spotted onto the center of each macrocarrier and allowed to dry about 2 minutes before bombardment.

[0143] The sample plates are bombarded at level #4 in a particle gun. All samples receive a single shot at 650 PSI, with a total of ten aliquots taken from each tube of prepared particles/DNA.

[0144] Following bombardment, the embryos are kept on 560Y medium for 2 days, then transferred to 560R selection medium containing 3 mg/liter Bialaphos, and subcultured every 2 weeks. After approximately 10 weeks of selection, selection-resistant callus clones are transferred to 288J medium to initiate plant regeneration. Following somatic embryo maturation (2-4 weeks), well-developed somatic

embryos are transferred to medium for germination and transferred to the lighted culture room. Approximately 7-10 days later, developing plantlets are transferred to 272V hormone-free medium in tubes for 7-10 days until plantlets are well established. Plants are then transferred to inserts in flats (equivalent to 2.5" pot) containing potting soil and grown for 1 week in a growth chamber, subsequently grown an additional 1-2 weeks in the greenhouse, then transferred to classic 600 pots (1.6 gallon) and grown to maturity.

[0145] Plants are monitored and scored for the appropriate marker, such as the control of a Coleoptera plant pest, such as a *Diabrotica* plant pest and have insecticidal activity. For example, R_0 plant roots are fed to western corn rootworm larvae (WCR, *Diabrotica virgifera*). Transgenic corn roots are handed-off in Petri dishes with MSOD medium containing antibiotics and glyphosate for in vitro selection. Two WCR larvae are infested per root in each dish with a fine tip paintbrush. The dishes are sealed with Parafilm to prevent the larvae from escaping. The assays are placed into a 27° C., 60% RH Percival incubator incomplete darkness. Contamination and larval quality are monitored. After six days of feeding on root tissue, the larvae are transferred to WCR diet in a 96 well plate. The larvae are allowed to feed on the diet for eight days making the full assay fourteen days long. Larval mass and survivorship are recorded for analysis. A one-way ANOVA analysis and a Dunnett's test is performed on the larval mass data to look for statistical significance compared to an untransformed negative control. WCR larvae stunting is measured after feeding on two events and compared to growth of larvae fed on negative control plants.

[0146] In other assays, transgenic corn plants (R_0) generated are planted into 10-inch pots containing Metromix soil after reaching an appropriate size. When plants reach the V4 growth stage, approximately 1000 Western corn rootworm (WCR, *Diabrotica virgifera*) eggs are infested into the root zone. Non-transgenic corn of the same genotype is infested at a similar growth stage to serve as a negative control. Eggs are pre-incubated so hatch occurs within 24 hours of infestation. Larvae are allowed to feed on the root systems for 3 weeks. Plants are removed from the soil and washed so that the roots can be evaluated for larval feeding. Root damage is rated using a Node Injury Scale (NIS) to score the level of damage where a 0 indicates no damage, a 1 indicates that one node of roots is pruned to within 1.5 inches, a 2 indicates that 2 nodes are pruned, while a 3 indicates that 3 nodes are pruned. Because the plants being used for evaluation are directly out of tissue culture after transformation and because transformation events are unique, only a single plant is evaluated per event at this time. The plants in the assay that present signs or symptoms of larval feeding indicate that a successful infestation is obtained. Negative control plant roots are moderately to severely damaged averaging whereas roots of the transgenic plants provide substantial control of larval feeding, with about 0.2 or less on the Node Injury Scale.

[0147] Bombardment medium (560Y) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000× SIGMA-1511), 0.5 mg/l thiamine HCl, 120.0 g/l sucrose, 1.0 mg/l 2,4-D, and 2.88 g/l L-proline (brought to volume with D-I H_2O following adjustment to pH 5.8 with KOH); 2.0 g/l Gelrite (added after bringing to volume with D-I H_2O); and 8.5 mg/l silver nitrate (added after sterilizing the medium and cooling to room temperature). Selection medium (560R) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000× SIGMA-1511), 0.5 mg/l thiamine HCl, 30.0 g/l sucrose, and 2.0 mg/l 2,4-D (brought to volume with D-I H_2O following adjustment to pH 5.8 with KOH); 3.0 g/l Gelrite (added after bringing to volume with D-I H_2O); and 0.85 mg/l silver nitrate and 3.0 mg/l bialaphos (both added after sterilizing the medium and cooling to room temperature).

[0148] Plant regeneration medium (288J) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g nicotinic acid, 0.02 g/l thiamine HCl, 0.10 g/l pyridoxine HCl, and 0.40 g/l glycine brought to volume with polished D-I H_2O) (Murashige and Skoog (1962) *Physiol. Plant.* 15:473), 100 mg/l myo-inositol, 0.5 mg/l zeatin, 60 g/l sucrose, and 1.0 ml/l of 0.1 mM abscisic acid (brought to volume with polished D-I H_2O after adjusting to pH 5.6); 3.0 g/l Gelrite (added after bringing to volume with D-I H_2O); and 1.0 mg/l indoleacetic acid and 3.0 mg/l bialaphos (added after sterilizing the medium and cooling to 60° C.). Hormone-free medium (272V) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g/l nicotinic acid, 0.02 g/l thiamine HCl, 0.10 g/l pyridoxine HCl, and 0.40 g/l glycine brought to volume with polished D-I H_2O), 0.1 g/l myo-inositol, and 40.0 g/l sucrose (brought to volume with polished D-I H_2O after adjusting pH to 5.6); and 6 g/l bacto-agar (added after bringing to volume with polished D-I H_2O), sterilized and cooled to 60° C.

Example 6. Agrobacterium-Mediated Transformation of Maize

[0149] For *Agrobacterium*-mediated transformation of maize with a silencing element of the invention, the method of Zhao is employed (U.S. Pat. No. 5,981,840, and PCT patent publication WO98/32326; the contents of which are hereby incorporated by reference). Such a construct can, for example, express a long double stranded RNA of the target sequence set forth in table 1. Such a construct can be linked to the dMMB promoter. Briefly, immature embryos are isolated from maize and the embryos contacted with a suspension of *Agrobacterium*, where the bacteria are capable of transferring the polynucleotide comprising the silencing element to at least one cell of at least one of the immature embryos (step 1: the infection step). In this step the immature embryos are immersed in an *Agrobacterium* suspension for the initiation of inoculation. The embryos are co-cultured for a time with the *Agrobacterium* (step 2: the co-cultivation step). The immature embryos are cultured on solid medium following the infection step. Following this co-cultivation period an optional "resting" step is contemplated. In this resting step, the embryos are incubated in the presence of at least one antibiotic known to inhibit the growth of *Agrobacterium* without the addition of a selective agent for plant transformants (step 3: resting step). The immature embryos are cultured on solid medium with antibiotic, but without a selecting agent, for elimination of *Agrobacterium* and for a resting phase for the infected cells. Next, inoculated embryos are cultured on medium containing a selective agent and growing transformed callus is recovered (step 4: the selection step). The immature embryos are cultured on solid medium with a selective agent resulting in the selective growth of transformed cells. The callus is then regenerated into plants (step 5: the regeneration step), and calli grown on selective medium are cultured on solid medium to regener-

ate the plants. Assays for insecticidal activity can be performed as described above in Example, 5.

Example 7 Expression of Silencing Elements in Maize

[0150] The silencing elements are expressed in a maize plant as hairpins and the plant is tested for insecticidal activity against corn root worms.

[0151] Maize plants are transformed with Plasmids containing at least one polynucleotide disclosed herein and plants expressing the silencing elements are transplanted from 272V plates into greenhouse flats containing Fafard Superfine potting mix. Approximately 10 to 14 days after transplant, plants (now at growth stage V2-V3) are transplanted into treepots containing Fafard Superfine potting mix. At 14 days post greenhouse send date, plants are infested with 100 eggs of western corn root worms (WCRW)/plant. For later sets, a second infestation of 100 eggs WCRW/plant is done 14 days after the first infestation and scoring is at 14 days after the second infestation. 21 days post infestation, plants are scored using CRWNIS. Those plants with a score of ≤0.5 are transplanted into large pots containing SB300 for seed.

[0152] The sequences referred to herein, SEQ. ID NOs: 1-723 are filed concurrently herewith in a textfile and are incorporated herein in their entireties.

[0153] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[0154] All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0155] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

The patent application contains a lengthy sequence listing. A copy of the sequence listing is available in electronic form from the USPTO web site (https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20240002842A1). An electronic copy of the sequence listing will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. An isolated polynucleotide comprising a nucleotide sequence comprising:

(a) the nucleotide sequence comprising any one of SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, wherein said polynucleotide encodes a silencing element having insecticidal activity against a Coleoptera plant pest.

(b) the nucleotide sequence comprising at least 9000 sequence identity to any one of nucleotides SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132,

133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, wherein said polynucleotide encodes a silencing element having insecticidal activity against a Coleoptera plant pest; or

(c) the nucleotide sequence comprising at least 19 consecutive nucleotides of any one of SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof, wherein said polynucleotide encodes a silencing element having insecticidal activity against a Coleoptera plant pest.

2. The isolated polynucleotide of claim **1**, wherein said Coleoptera plant pest is a *Diabrotica* plant pest.

3. An expression cassette comprising the polynucleotide of claim **1**.

4. The expression cassette of claim **3**, wherein said polynucleotide is operably linked to a heterologous promoter.

5. The expression cassette of claim **3**, wherein said polynucleotide is expressed as a double stranded RNA.

6. The expression cassette of claim **3**, wherein said polynucleotide comprise a silencing element which is expressed as a hairpin RNA.

7. The expression cassette of claim **6**, wherein the silencing element comprises, in the following order, a first segment, a second segment, and a third segment, wherein

a) said first segment comprises at least about 19 nucleotides having at least 90% sequence complementarity to a target sequence set forth in any one of SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613,

614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof;

b) said second segment comprises a loop of sufficient length to allow the silencing element to be transcribed as a hairpin RNA; and,

c) said third segment comprises at least about 19 nucleotides having at least 85% complementarity to the first segment.

8. The expression cassette of claim **3**, wherein said polynucleotide is flanked by a first operably linked convergent promoter at one terminus of the polynucleotide and a second operably linked convergent promoter at the opposing terminus of the polynucleotide, wherein the first and the second convergent promoters are capable of driving expression of the polynucleotide.

9. A host cell comprising a heterologous expression cassette of claim **3**.

10. A plant cell having stably incorporated into its genome a heterologous polynucleotide comprising a silencing element, wherein said silencing element comprises

a) a fragment of at least 19 consecutive nucleotides of any one SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof; or,

b) the nucleotide sequence comprising at least 90% sequence identity to any one of SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof;

wherein said silencing element, when ingested by a Coleoptera plant pest, reduces the level of a target sequence in said Coleoptera plant pest and thereby controls the Coleoptera plant pest.

11. The plant cell of claim **10**, wherein the Coleoptera plant pest is a *Diabrotica* plant pest.

12. (canceled)

13. The plant cell of claim **10**, wherein said plant cell comprises the expression cassette of claim **8**.

14. The plant cell of claim **10**, wherein said silencing element expresses a double stranded RNA.

15. The plant cell of claim **10**, wherein said silencing element expresses a hairpin RNA.

16. The plant cell of claim **15**, wherein said polynucleotide comprising the silencing element comprises, in the following order, a first segment, a second segment, and a third segment, wherein

a) said first segment comprises at least about 19 nucleotides having at least 90% sequence complementarity to a target sequence set forth in any one of SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof;

b) said second segment comprises a loop of sufficient length to allow the silencing element to be transcribed as a hairpin RNA; and,

c) said third segment comprises at least about 19 nucleotides having at least 85% complementarity to the first segment.

17. The plant cell of claim **10**, wherein said silencing element is operably linked to a heterologous promoter.

18. (canceled)

19. (canceled)

20. (canceled)

21. (canceled)

22. (canceled)

23. (canceled)

24. A method for controlling a Coleoptera plant pest comprising feeding to a Coleoptera plant pest a composition comprising a silencing element, wherein said silencing element, when ingested by said Coleoptera plant pest, reduces the level of a target Coleoptera plant pest sequence and thereby controls the Coleoptera plant pest, wherein said target Coleoptera plant pest sequence comprise a nucleotide sequence comprising at least 90% sequence identity to any one of SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof.

25. The method of claim **24**, wherein said Coleoptera plant pest comprises a *Diabrotica* plant pest.

26. The method of claim **24**, wherein said silencing element comprises

a) a fragment of at least 19 consecutive nucleotides of any one SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133, 136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392,

393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof; or,

b) a nucleotide sequence comprising at least 9000 sequence identity to any one of SEQ ID NOS: 1, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, 44, 45, 48, 49, 52, 53, 54, 55, 56, 57, 60, 61, 64, 65, 68, 69, 72, 73, 76, 77, 80, 81, 84, 85, 88, 89, 92, 93, 96, 97, 100, 101, 104, 105, 108, 109, 112, 113, 116, 117, 120, 121, 124, 125, 128, 129, 132, 133,136, 137, 140, 141, 144, 145, 148, 149, 152, 153, 156, 157, 160, 161, 164, 165, 168, 169, 172, 173, 176, 177, 180, 181, 184, 185, 188, 189, 192, 193, 196, 197, 200, 201, 204, 205, 208, 209, 212, 213, 216, 217, 220, 221, 224, 225, 228, 229, 232, 233, 236, 237, 240, 241, 244, 245, 248, 249, 252, 253, 256, 257, 260, 261, 264, 265, 268, 269, 272, 273, 276, 277, 280, 281, 284, 285, 288, 289, 292, 293, 296, 297, 300, 301, 304, 305, 308, 309, 312, 313, 316, 317, 320, 321, 324, 325, 328, 329, 332, 333, 336, 337, 340, 341, 344, 345, 348, 349, 352, 353, 356, 357, 360, 361, 364, 365, 368, 369, 372, 373, 376, 377, 380, 381, 384, 385, 388, 389, 392, 393, 396, 397, 400, 401, 404, 405, 408, 409, 412, 413, 416, 417, 420, 421, 424, 425, 428, 429, 432, 433, 436, 437, 440, 441, 444, 445, 448, 449, 452, 453, 456, 457, 460, 461, 464, 465, 468, 469, 472, 473, 476, 477, 480, 481, 484, 485, 488, 489, 492, 493, 496, 497, 500, 501, 504, 505, 508, 509, 512, 513, 516, 517, 520, 521, 524, 525, 528, 529, 532, 533, 536, 537, 540, 541, 544, 545, 548, 549, 552, 553, 556, 557, 560, 561, 562, 563, 564, 565, 566, 567, 568, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 700, 701, 702, 703, 706, 707, 708, 709, 712, 713, 714, 715, 718, 719, 720, 721, or active variants and fragments thereof, and complements thereof.

27. The method of claim **25**, wherein said *Diabrotica* plant pest comprises *D. virgifera virgifera, D. virgifera zeae, D. speciosa, D. barberi, D. virgifera zeae, D. speciosa*, or *D. undecimpunctata howardi.*

28. (canceled)

29. (canceled)

30. The method of claim **24**, wherein said silencing element expresses a double stranded RNA.

31. The method of claim **24**, wherein said silencing element comprises a hairpin RNA.

32. The method of claim **31**, wherein said polynucleotide comprising the silencing element comprises, in the following order, a first segment, a second segment, and a third segment, wherein

a) said first segment comprises at least about 19 nucleotides having at least 90% sequence complementarity to the target polynucleotide;

b) said second segment comprises a loop of sufficient length to allow the silencing element to be transcribed as a hairpin RNA; and,

c) said third segment comprises at least about 19 nucleotides having at least 85% complementarity to the first segment.

33. (canceled)

34. (canceled)

35. (canceled)

36. (canceled)

37. (canceled)

38. (canceled)

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