PROJECT FINAL REPORT

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Project acronym: EcoSyn

Project title: Ecofriendly synergists for insecticide formulations

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4.1 Final publishable summary report

This section must be of suitable quality to enable direct publication by the Commission and should preferably not exceed 40 pages. This report should address a wide audience, including the general public.

The publishable summary has to include **5 distinct parts** described below:

- **An executive summary** (not exceeding 1 page)

The project, (EcoSyn), was established to identify and develop synergists for insecticide formulations in both agricultural and public health applications, enabling deployment strategies that enhance the effectiveness of several important classes of insecticide.

An important aim was to enable a reduction in the amount of insecticidal active applied whilst maintaining efficacy, thereby reducing any adverse effects of these insecticides on beneficial insects such as bees. As such, the novel synergists may be defined as eco-friendly.

The project duration was 27 Months

Eight participants, - from Italy (2), - United Kingdom (3), –Hungary (1), - Czech Republic (1) and Turkey (1) have contributed to the project.

Three SMEs were represented (Italy, UK, Hungary), three RTDs (Italy, UK, Czech Republic) and two Others (UK and Turkey).

A Consortium Agreement was signed between the Parties where the IP protection and Foreground was specifically mentioned and agreed. On this basis reference papers were published and attendance at several international thematic Conferences was promoted.

From the works carried out by all the participants and in accordance with the objectives of the project, two novel synergists, viz. EN1-218 particularly suitable for agro application and EN1-216 for public health were identified.

Both synergists enable a reduction of up to ½ the field rate dose of the insecticides and can therefore be termed eco-friendly.

Particular attention was focused on costs, to maintain commercial viability -one aim of the project.

Considering IP, three patent applications were filed and in accordance with the Consortium Agreement rules, the applicants were the three SMEs.

- **A summary description of project context and objectives** (not exceeding 4 pages) -

Insect pests represent a threat to agricultural production and the health of humans and domestic animals through crop damage and vector transmission of several important diseases. The economic impact of insect pests is huge in terms of human and animal health - as well as for agricultural production.

Chemical insecticides have been, and remain, the mainstay for controlling insect pests and represent a guarantee for the supply of affordable food as well as a tool for an effective disease vector control for the foreseeable future. Unfortunately, the world-wide use of high levels of synthetic insecticides to control insect pests over many years has led to increased selection for insecticide resistance and has contributed to environmental contamination.

Resistance leads to wasteful and ineffective insecticide treatments and may prompt a return to chemicals with less favourable environmental profiles as used in the past. Central to these issues is the emerging problem of how to meet the challenges of a growing population by producing more food whilst minimising negative environmental impacts (sustainable intensification). For both crop protection and vector control this needs to be achieved without a simultaneous escalation in insecticide usage.

The World Health Organisation (WHO) defines resistance as “the inherited ability of a strain or organism to survive doses of a toxicant that would kill the majority of individuals in a normal population of the same species”

Resistance to insecticides most commonly evolves through two main mechanisms;

1) enhanced production of metabolic enzymes that break down or sequester the insecticide before it reaches the target protein (termed metabolic resistance)
2) mutation of the insecticide target protein resulting in a loss or reduction in insecticide binding (termed target-site resistance). These mechanisms may exist individually in an insect or may be found in combination where the overall resistance they confer is substantially greater.

Three main classes of enzymes have been shown to be involved in the detoxification of insecticides and metabolic resistance. These are esterases, cytochrome P450s (P450s) and glutathione-S-transferases (GSTs)

Esterases and P450s are involved in phase 1 detoxification as they can act on the intact insecticide, whereas GSTs are phase 2 enzymes, acting on degradation products of phase 1 enzyme activity. Synergists can increase the efficacy of insecticides by inhibiting one or more of these metabolic enzyme families. Because both resistant and susceptible insects express these enzymes, inhibition can make susceptible populations hyper-sensitive to insecticides in addition to overcoming the resistance of insects that over express these enzymes.

PBO (piperonyl butoxide) is a well-known synergist and is widely used in the household market. As yet, it has only a niche market in agriculture. PBO is now commonly applied with pyrethrins, synthetic pyrethroids and many other insecticidal actives. Many studies in the literature report on the mechanisms of action of PBO, initially as an inhibitor of cytochrome P450s and subsequently of resistance-associated esterases. Currently, progress has been made regarding the interaction of PBO with a resistance-associated esterase (E4) and with the major human detoxifying P450, CYP3A4. However, such studies have not been carried out on recombinant enzymes known to be responsible for conferring insecticide resistance. Furthermore, there have been no studies to investigate the effect of selection by the use of a synergist in combination with a decreased insecticide dosage compared with selection of insecticide at the field rate.

In terms of advancing knowledge and technical progress, the project is aimed to clarify the interaction of PBO (as the standard product) with the insect defence enzymes. On the basis of these interactions, to define and model potentially more potent structures, with a view to implement eco-friendly synergists for use with insecticides in both agriculture and Public Health applications through the following steps:

- Elucidation of the mechanism of action of the standard synergist, PBO, widely in current use in household and PCO applications.
- Design of novel synergists based on experimental results, SAR of PBO and existing analogues, molecular modelling whilst maintaining important physico-chemical parameters.
- Choice of appropriate agriculturally-important susceptible and resistant pest insects
- Examples of beneficial insects found in the same environment.
- Choice of appropriate susceptible and resistant disease vector insects (Public Health).
- Choice of appropriate insecticide actives for evaluating synergistic effects on susceptible and resistant populations of economically important insect pests.
- Evaluation of the activity on pest and beneficial species using laboratory bioassays and field trials.
- Evaluation of the potential of synergist/insecticide application to select for resistance compared with application of insecticide alone.
- Process development to obtain the definitive synergist(s) with the aim of achieving an industrial and economically viable process.
- Definition of global patent rights to protect the invention.

A further objective of the project was the promotion of the novel synergists in combination with reduced levels of insecticidal actives as part of an integrated pest management strategy.

As the aim of the project involved selection of possible synergists for commercial opportunity, consideration was based on the cost performance ratio, as well as synthesis pathways.

The project was structured on this basis in seven different work packages (WP) as follows:

- ✓ **Elucidation of the mechanism of action of the standard synergist PBO and existing analogues using purified or recombinant P450 and** subsequent interactions through a structure-activity relationship (SAR) between the purified enzymes and piperonyl butoxide and existing analogues **(WP1 and WP2)**
- ✓ **Design of novel synergists based on experimental results, SAR, molecular modelling and their synthesis.** A**s** one aim of the project was consideration of commercial opportunity, a preliminary cost estimation was taken into consideration **(WP3)**
- ✓ **Evaluation of the efficacy of the novel synergists on pest and beneficial species using laboratory bioassays (WP4)**

The evaluation was carried out on agriculturally important pests such as *Myzus persicae (M. persicae)* (green peach potato aphid), *Bemisia tabaci (B. tabaci)* (cotton whitefly), *Meligethes aeneus (M. aeneus)* (pollen beetle) and on beneficial insects such as *Apis mellifera (A. mellifera)* and *Bombus terrestris (B. terrestris).*

For vector disease (public health) insects, *Blattella germanica (B. germanica)* (German cockroach) and *Musca domestica (M. domestica)* (common housefly) were used.

To evaluate synergistic effects on susceptible and resistant populations of economically important insect pests, different classes of insecticide with differing modes of action and application were considered.

- Cypermethrin a pyrethroid
- Tau fluvalinate a pyrethroid
- S-Methoprene an IGR
- Imidacloprid a neonicotinoid
- Thiacloprid a neonicotinoid

Due to restriction of neonicotinoid application in some countries such as Turkey, thiamethoxam was also included

- ✓ **Evaluation of the activity of the novel synergists on pest and beneficial species using glasshouse and semi-field trials (WP5)**

The evaluation was carried out on the basis of a chosen range of the novel synergists in semi-field conditions and by using the same insecticides as mentioned above.

- ✓ **Evaluation of the potential of synergist/insecticide applications to select for resistance (WP6)**

Field populations of *B.tabaci* and *M. persicae* were collected and bioassayed with a pyrethroid and a neonicotinoid in combination with PBO, providing selected and unselected strains for molecular characterisation. Further characterisation of the change in gene expression between the selected and unselected pools and between those selected with insecticide alone or insecticide+PBO can be used to evaluate whether a regime incorporating a synergist increases selection for single, or multiple, resistance genes.

- ✓ **Validation and Process development to obtain the definitive synergist(s) (WP7)**

The novel synergists identified on the basis of the tests carried out in WP4 and in WP5 were validated. Furthermore, a development study was carried out to confirm the chemical feasibility from an industrial point of view, taking into consideration the costs of the intermediates as well as the safety of different chemical steps.

Another important point for consideration was the patentability of the novel synergist(s).

This was agreed by all SMEs, in accordance with the Consortium Agreement signed among the participants.

Two other work packages were utilised; **Dissemination of the results** (**WP8)** and the **Project management (WP9)**

- **A description of the main S&T results/foregrounds** (not exceeding 25 pages)

The project was divided in nine work packages which are described below, together with results obtained.

WP1

The project began with the isolation and purification of specific metabolic enzymes (esterases and P450s) that have been reported to be responsible for conferring resistance to insecticides in economically important crop pests.

The fine detail of WP1 is reported in Deliverable 1.1 and is only summarized here.

The esterases were prepared from a bulk collection of insects via purification, whilst the P450s were prepared recombinantly (CYP6CM1 and CYP6CY3). For the latter, reduced CO-difference spectrum revealed a classic Soret peak for both CYP6CY3 and CYP6CM1 at 450 nm indicative of a stable, good quality functional enzyme. In initial enzyme assays of P450 expressed using Sf9 cells activity was demonstrated for both P450s although greater O-dealkylation activity of two fluorescent model substrates MFC (7-Methoxy-4-(tri-fluoromethyl) coumarin) and EFC (7-ethoxy-4-trifluoro-methylcoumarin) was revealed for CYP6CY3 than CYP6CM1.

When the expression of CYP6CM1/CYP6CY3 was repeated using the Tn5 cell line from *Trichoplusia ni* (Lepidoptera: Noctuidae) the specific activity of the microsomal preparations was significantly enhanced. Therefore these preparations were selected for use in the subsequent WP2 with the aim to assess the activity of the novel synergists. Purification of esterase was successful, with enzyme assays using 1-naphthyl acetate as substrate confirming high quality functional esterase had been obtained.

WP2

The aim of WP2 was to assess the inhibitory potency of the novel synergists against P450s and esterase(s) from WP1. The details of this are reported in Deliverable 2.1, so only a summary is supplied here.

Inhibition of the insect defence enzymes was achieved by monitoring the decrease in activity against model substrates (7-ethoxycoumarin (7-EC) for CYP6CM1 and 7-ethoxy-4-trifluoromethylcoumarin (EFC) for CYP6CY3; 4-nitrophenyl acetate (pNA) for FE4 and 4-nitrophenyl octanoate (pNO) for *B. tabaci* esterases).

Each enzyme / inhibition SAR delivered its own bespoke predictions for increased potency as tabulated in Deliverable 2.1. Thus, no single structure could be predicted as the 'ultimate' structure to confer highest inhibition against all enzymes, but rather it would depend upon the nature of the resistance conferred (i.e. predominately P450-based etc).

However, the SAR supplied evidence that alkynyl structures with particular alkyl chain length would be the basic structural template to confer high inhibition.

Table 1 below shows the sum of activity remaining for CYP6CM1, CYP6CY3 and FE4 after treatment by the potential novel synergists in comparison with the commercial synergist piperonyl butoxide.

Enzyme activity remaining (%) CYP6CM1 + enzyme remaining (%) CYP6CY3+ enzyme activity remaining (%) FE4 = total activity remaining given in Table 1. If no inhibition occurred, the total would be 300.

Table 1. Remaining activities for CYP6CM1, CYP6CY3 and FE4

Synergist internal code	Total activity remaining
1-215	44.45
16-41	59.07
16-46	60.28
1-126	70.21
16-51	75.13
1-216	86.26
16-40	90.83
16-42	96.21
1-219	96.78

1-213	97.22
1-223	100.4
1-218	116.8
PBO (comparison)	162.9

The first 4 structures are the same basic template, predicted by WP2 to confer highest inhibition (and thus synergism).

Protein modelling *in silico* was completed using closest templates from the PDB database and Autodock vina. No assumptions were made about the docking location of the ligand, the grid box encompassing the whole protein.

It was found that docking locations were in agreement with locales reported to be responsible for metabolism of insecticides from the literature i.e. serine 387 and arginine 224 in *B. tabaci*.

Binding affinities (free energy) of the potential novel synergists were in agreement with the inhibition potency.

WP3

The aim of WP3 was to synthesize the potential novel synergists on the basis of the SAR evaluation and *in-silico* modelling. The activities were carried out in the laboratory at the gram scale in close cooperation with the leader of WP2. In this way the work was developed as an interactive effort to identify the optimal products based on *in vitro* analysis. At the same time a literature search was carried out to confirm the originality and chemical feasibility of the potential novel synergists.

The details of the activities of WP3 are reported in Deliverable 3.1 and 3.2, so only a summary is supplied here. The potential novel synergists had structures basically attributable to three chemical families: benzodioxole derivatives containing a triple bond, benzodioxole derivatives containing an alkoxyalkyl chain and 2,3-dihydro benzofurane derivatives. The literature search suggested that most of the products were novel and potentially patentable. All of the structures were checked using two different techniques, viz MS and ^{1}H and ^{13}C NMR. Particular attention was given to 2, 3-dihydrobenzofurane derivatives where the definition of positional isomers was required. In this instance, the analyses were carried out through gCOSY NMR and verified, where possible, through X- rays. All the products were checked for their activity *in vitro* as reported in WP2 and particular attention was given to the synthetic and economic feasibility of the products as potential novel synergists, taking into account the cost/performance ratio to be assessed *in vitro* and subsequently validated *in vivo*. A main objective of the project was to identify potential novel synergists to be used in agro applications, and the chemical process cost evaluation was a key factor for their -potential implementation.

WP4

WP4 used laboratory bioassays to assess *in vivo* efficacy of pyrethroids (cypermethrin/tau-fluvalinate), neonicotinoids (imidacloprid/thiacloprid) or IGR (S-Methoprene), alone or in combination with PBO/novel synergists, on different populations of important agricultural and household pests (*M. persicae*, *B. tabaci*, *M. aeneus*, *B. germanica* and *M. domestica*) possessing various levels of resistance to insecticides.

Simultaneously, laboratory bioassays evaluated the effects of the above products on honeybees / bumblebees. A detailed report of the methodology and results of this WP are provided as Deliverable 4.1.

A summary of the activity is given below.

One susceptible (1X) and two resistant clones (96H and 92H6) of *M. persicae* (aphids*)* were used in bioassays: the latter originated from individual parthenogenetic females from field populations collected in Italian peach orchards in 2010 (92H6) and 2011 (96H) after neonicotinoid and pyrethroid application failures. A highly resistant population of *B. tabaci (*whitefly) was collected from ornamental plants in glasshouses in Northern Italy after control failures with several insecticide classes. *Meligethes aeneus* (pollen beetle) populations were collected in Northern Italy and Poland.

In assays using *M. domestica* (house fly) two internal standard populations with different levels of insecticide susceptibility were included.

All bioassays used commercially available formulations of alpha-cypermethrin (Fastac), tau-fluvalinate (Mavrik 20 EW), imidacloprid (Confidor 200 SL), thiacloprid (Calypso), cypermethrin (Cymina) and S-methoprene (Biopren 20EC).

Bioassay methodology reflected the combination of pest, insecticide, synergist and endpoint. A baseline for each combination of pest and insecticide was determined and a diagnostic dose conferring 25-35% mortality was used in subsequent tests to identify synergism levels of the novel synergists.

Preliminary bioassays were conducted using both technical grade and EC formulated synergists (PBO and EN1-126 (EN126) with split applications.

Subsequently, EC formulated synergists only were used in tank-mix application against resistant clones. The EC formulation alone was not toxic.

M. persicae

Occasional high mortality produced by EN1-213 alone was observed in clone 96H, whilst in clone 92H6 high mortality was produced by EN1-218 and EN1-126. Synergistic ratios (Insecticide + synergist efficacy / Insecticide alone efficacy) recorded in these tests are reported in Table 2.

Table2. *M. persicae*. Synergistic ratios calculated for a discriminating dose of the selected insecticides and EC formulated novel synergists, applied at 1 g /L, without split application (tank mix), against two resistant clones of *M. persicae*. Darker cells represent higher synergistic ratio values.

	96H				92H6			
	Confidor	Calypso	Fastac	Mavrik	Confidor	Calypso	Fastac	Mavrik
EN1-126	1.4	2.1	-1.9	0.7	6.7	2.5	14.1	27.3
EN1-213	1.0	2.0	5.1	0.8	6.3	2.4	7.0	33.1
EN1-215	1.3	2.0	-3.6	1.1	6.3	2.5	7.1	46.8
EN1-216					5.1	2.6	21.7	12.8
EN1-218					5.0	2.6	11.1	8.0
EN1-219	1.3	0.9	-0.3	0.8				
PBO	1.2	2.1	0.5	0.7	5.7	2.1	0.0	0.0

Mortality data (percentage arcsin transformed) were statistically analysed using analysis of variance (ANOVA). Significant differences were observed in neonicotinoid treatments with clone 96H.

All the Calypso + synergist mixtures (except EN1-219) produced significantly higher mortality than Calypso alone.

In bioassays using Confidor, only the mix with EN1-126 was statistically different from Confidor alone. No significant differences were observed with Fastac. In tests with Mavrik significant differences were present only between mixtures of the insecticide plus synergists and synergists alone. Mixtures were not different from insecticide alone.

In bioassays with clone 92H6 and neonicotinoid mixtures, insecticide plus synergist were significantly different from insecticide and synergist alone. No significant differences were observed for Fastac. With Mavrik, EN1-215 was the most effective synergist.

B.tabaci

Synergistic ratios (Insecticide + synergist efficacy / Insecticide alone efficacy) calculated from tests against *B. tabaci* are reported in Table 3.

Mortality data (percentage arcsin transformed) were statistically analysed using analysis of variance (ANOVA). Mortality observed in whiteflies treated with synergists alone was low and not statistically different from that observed in the untreated group. Highest mortality was observed in those groups treated with insecticide+synergist, but this was not always significantly different from insecticide alone.

Table3. *B. tabaci*. Synergistic ratios calculated for a discriminating dose of the selected insecticides and technical grade novel synergists in acetone, applied at 0.5 /L, 5 hours before insecticide application, against a resistant population of *B. tabaci*. Darker cells represent higher synergistic ratio values.

	Alb - resistant			
	Confidor	Calypso	Fastac	Mavrik
EN1-126	0.75	1.51	1.80	1.03
EN1-213	0.85	1.11	1.35	1.50
EN1-215	0.83	1.45	1.54	1.33
EN1-216	0.76	1.57	1.39	1.57
EN1-219	0.71	0.81	0.71	0.85
PBO	1.22	1.54	2.03	1.38
EN16-41	1.26	1.67	1.75	1.53

As with *M. persicae* it was decided to adopt a tank mix bioassay using EC formulated synergists. Again, the number of synergists to be assayed was restricted according to results from the *in vitro* assays.

The synergists were used at two different rates: 0.5 g/L and 1 g/L. The products were applied using a Potter spray tower. An increase in efficacy as well as in synergistic ratios was observed when the synergist application rate was increased (Table 4).

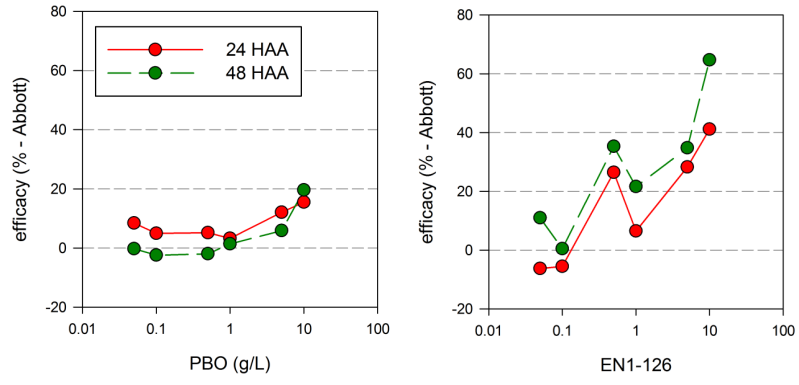
Table 4. *B. tabaci*. Synergistic ratios calculated for a tank mix of a discriminating dose of the selected insecticides and EC formulated synergists applied at 0.5 and 1 g/L, against a resistant population. Darker cells represent higher synergistic ratio values.

Synergist:	*0.5 g/L*				*1 g/L*			
	Confidor	Calypso	Fastac	Mavrik	Confidor	Calypso	Fastac	Mavrik
EN1-126	2.59	1.14	1.16	1.58	2.16	3.87	4.20	0.54
EN1-213	1.70	1.23	1.81	1.22	2.37	2.86	5.69	1.09
EN1-215	1.85	1.37	1.99	1.29	2.55	3.58	5.29	0.80
EN1-216					0.36	1.52	2.63	0.45
EN1-218					1.60	3.30	5.20	1.23
EN1-219	1.30	0.48	0.21	0.76	1.01	2.02	1.86	1.36
PBO	2.19	0.68	2.92	1.70	1.19	2.28	4.91	0.66

Baseline estimations of synergist toxicity

Baselines of synergist only toxicity against peach-potato aphids were estimated using one susceptible (1X) and one resistant (92H6) clone. Results are shown below. When applied against the susceptible clone, PBO (at concentrations 0.05 g/L to 10 g/L) gave only low toxicity). The standard concentration (1 g/L) was non-toxic for PBO, EN1-216 and EN1-218. The highest toxicity was recorded with EN1-126 and EN1-215 at 10 g/L (Figure 1).

All synergists (at concentrations up to 5 g/L) used against the resistant clone (92H6) gave low mortality. The highest toxicity was observed for EN1-126, EN1-215 and EN1-216 at 10 g/L (Figure 2).



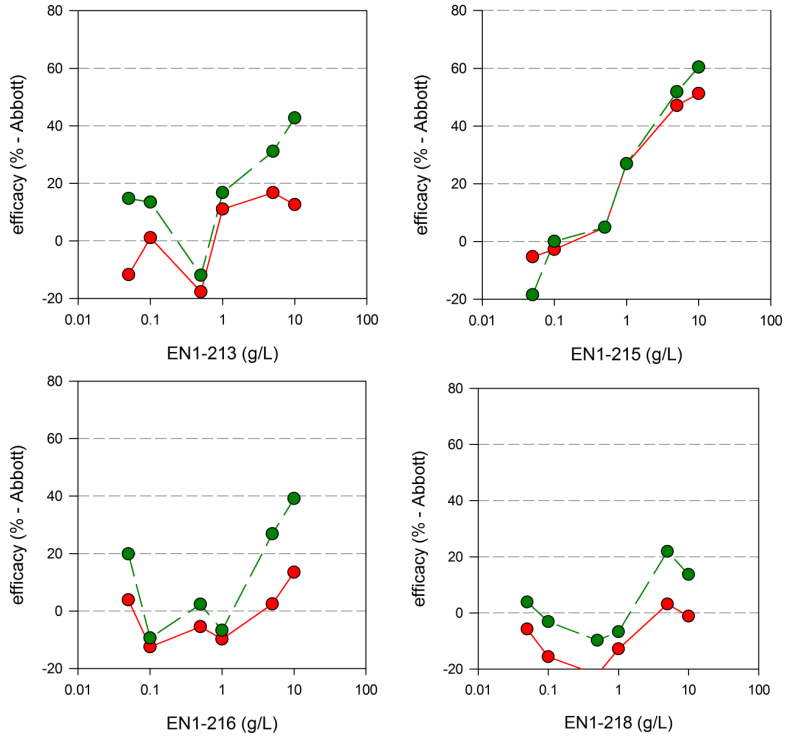
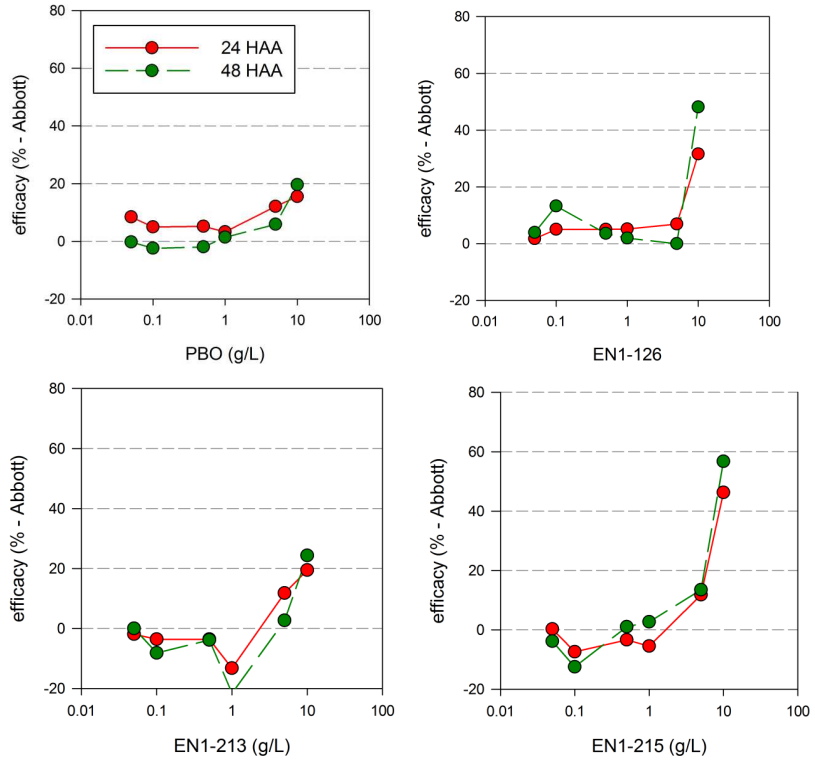


Figure 1. *M. persicae*. Baseline efficacy of EC formulated novel synergists applied in a dip-test bioassay against a susceptible clone (1X).



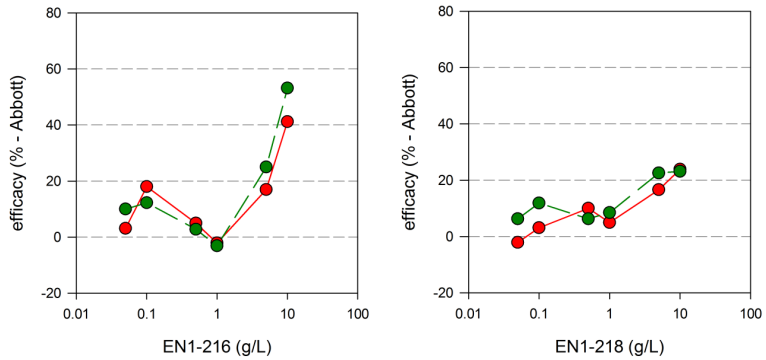


Figure 2. *M. persicae*. Baseline efficacy of EC formulated novel synergists applied in a dip-test bioassay against a resistant clone (92H6).

Meligethes aeneus

As no laboratory rearing protocol exists for this species, field collected populations were used in vial bioassays.

Populations were collected from Northern Italy and Poland in spring 2014 and 2015.

The only novel synergist tested in 2014 was EN1-215, at concentrations of 0.1 and 0.01 g/L., PBO and EN1-126 were also tested, for comparison.

Synergists alone, at a concentration of 0.1 g/L, were toxic and so only the 0.01 g/L dose was used. Considering the three Polish populations collected in 2014, PBO synergised pyrethroids in all three and neonicotinoids in population 1; whilst for populations 2 and 3 the best performance with neonicotinoids was achieved with EN1-215 .

In 2015 an Italian population (Table 5) was tested and the highest synergistic ratios were observed with Confidor: EN1-126 was the most potent, followed by EN1-215 and PBO. Synergistic ratios were lower with Calypso, but the ranking was similar to that of Confidor. With pyrethroids the highest ratios were obtained by EN1-215/EN1-216 followed by EN1-126 when applied together with Fastac; with Mavrik the ranking was EN1-126/ PBO followed by EN1-215 .

Using a Polish population, the highest synergistic ratio was observed with Confidor: PBO, EN1-213 and EN1-215 were the most potent, but EN1-126 was the least toxic when used alone. Synergistic ratios were lower with Calypso, where EN1-126 produced the highest ratio as a consequence of its lower innate toxicity. Considering Fastac and Mavrik, all the synergists were equipotent, with higher synergistic ratios observed with the latter.

Table5. *M. aeneus*. (2015). Synergistic ratios calculated for discriminating doses and novel synergists in vial bioassays. Darker cells represent higher synergistic ratio values.

	Italy - 2015			
	Confidor	Calypso	Fastac	Mavrik
PBO	6.9	1.8	1.3	2.5
EN1-126	9.3	1.9	1.8	2.5
EN1-213	2.8	1.5	1.5	2.3
EN1-215	6.5	1.7	1.9	2.2
EN1-216	3.8	1.6	1.9	2.1
EN1-218	2.5	1.7	1.6	2.1

	Poland - 2015			
	Confidor	Calypso	Fastac	Mavrik
	12.5	1.4	1.8	3.2
	10.9	1.8	2.1	2.9
	12.1	1.9	2	3.3
	12.8	1.4	2	3.8
	10.2	1.7	1.9	3.6
	10	2	2	3.7

An estimation of innate synergist toxicity against Pollen Beetle from Italy was made. Results are plotted in Figure 3. Mortality, corrected with Abbott's formula, was usually below 20%. Although EN1-213 produced an increase in mortality when applied at 0.1 g/L (0.014 mg/cm^2), ANOVA confirmed there were no statistically significant differences between the doses for each product.

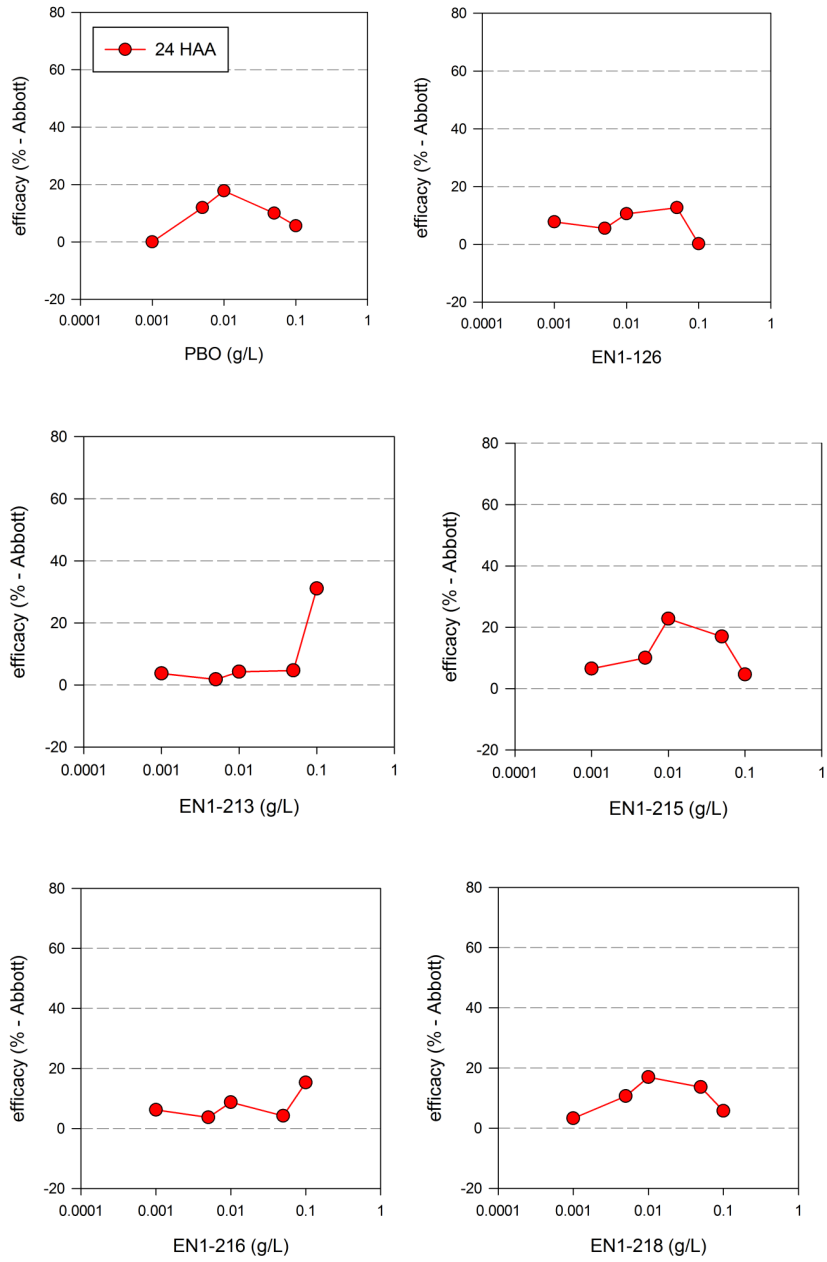


Figure 3. *M. aeneus*. Baseline efficacy of novel synergists applied in a vial bioassay against an Italian population collected near Piacenza.

Blattella germanica

Bioassays with Cypermethrin

Following the estimation of a dose-response for Cymina (cypermethrin) against *B. germanica* a discriminating dose of 120 µg (a.i.)/mL was applied together with 1 mg (a.i.)/mL of synergist.

Knock down was monitored every 5 minutes up to 30 minutes and mortality 24 hours later. Only EN1-213 produced an increase in knock down in comparison with the insecticide alone. Knock down produced by synergist alone was usually negligible (Figure 4). No toxic effects were observed with synergists alone: only EN1-213 produced a very low mortality. All the synergists mixed with Cymina produced a significant

increase in mortality 24 hours after the application in comparison with the synergists and the insecticide alone (Figure 5).

Mortality produced by the synergists alone was never statistically different from the mortality of the untreated control.

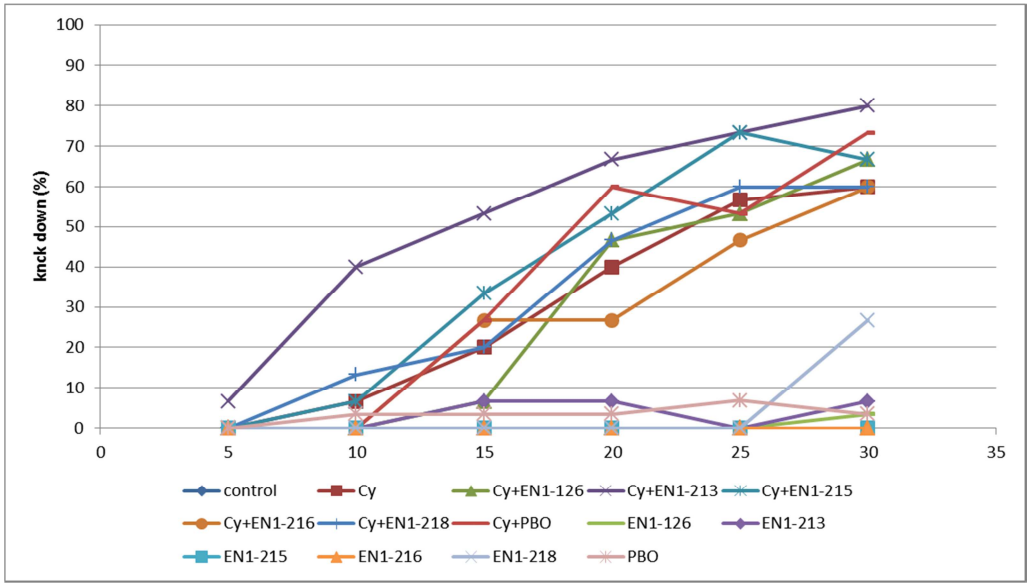


Figure 4. *B. germanica*. Knock down effect produced by a diagnostic dose of Cymina (cypermethrin) and novel synergists in the vial bioassay

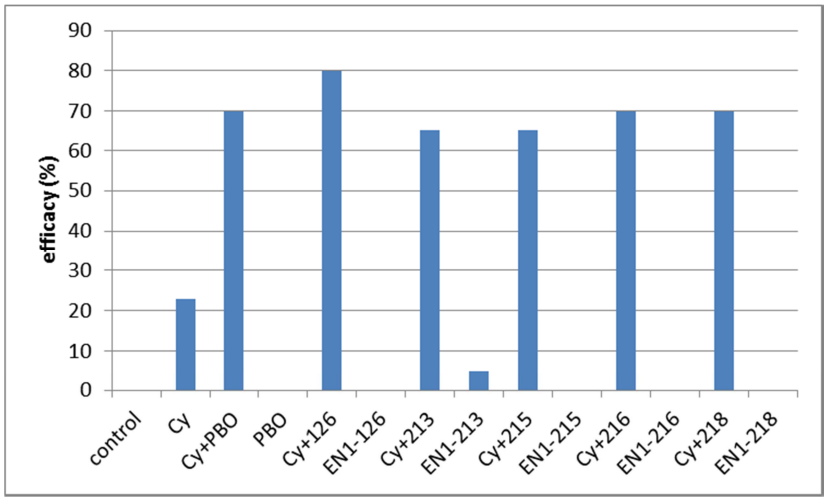


Figure 5. *B. germanica*. Mortality (efficacy according to Abbot's formula) produced by a diagnostic dose of Cymina (cypermethrin) and novel synergists in vial bioassay. Assessment made 24 hours after the application of the product. Contact time 30'.

Bioassays with S-methoprene (IGR)

S-methoprene is an active substance that mimics juvenile hormone, essential in the development of insects. When IGR formulations are tested, the development of the tested species and their offspring is monitored rather than mortality. The mechanism of action of an IGR is to arrest development of insects and their colonies. Thus, IGR trials have a long end-point, with mortality resulting from deformation and suffocation at the pupae stage.

To test the efficacy of S-methoprene against adults, a novel bioassay procedure was developed: single virgin females were isolated and fed for 7 days with approximately 1 mL of an artificial diet obtained by mixing 20% fish feed (powder), 0.25% S-methoprene (a.i.) and synergist used at 1:3 ratio and water to 100%. After 7 days females were removed and mixed with males for mating and checked daily for egg case development, abortion, hatching of larvae and female mortality. Mortality of females was quite low and no mortality was

observed in untreated control specimens. There was a significant reduction of offspring from treated females but this effect seemed to be transient and linked to the general reduction of fertile egg case production rather than a reduction of the vitality and number of eggs developing inside the ootheca produced by the treated female.

Egg case production was reduced by applying EN1-215 + methoprene and EN1-216 + methoprene. All the treatments induced abortion in the range of 40 to 80 % of the produced oothecae with the exception of EN1-216. The second egg case aborted from 20 to 100%. Often only 50% of egg cases produced viable larvae.

A statistically significant reduction in the total number of emerging larvae was observed with the first egg case (Figure 6). A delay in the production of the first egg case was observed compared to the untreated control, but not in the time between egg case production and hatching, nor the production and hatching of the second egg case.

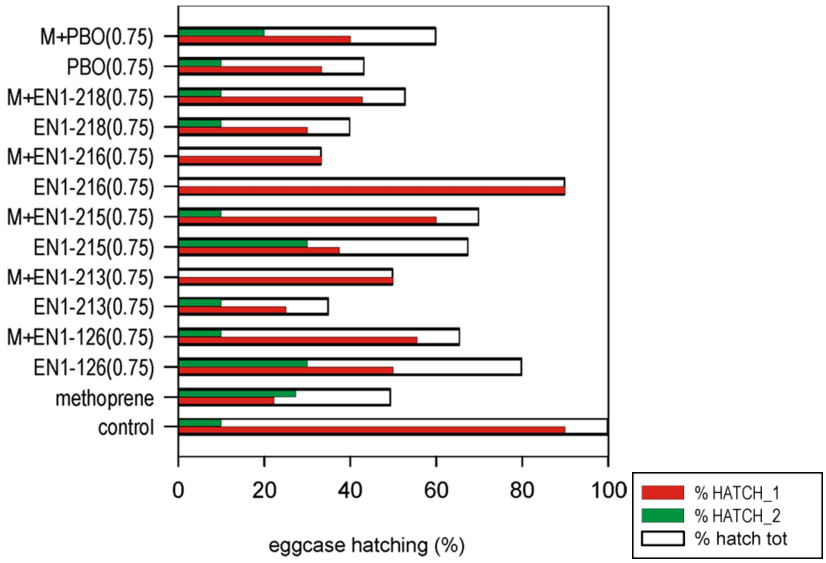
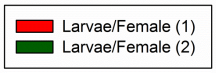


Figure 6. *B. germanica.* Eggcase hatching (%): first eggcase (% HATCH_1); second eggcase (% HATCH_2) and total (% hatch tot).



Neonicotinoids

Tests with imidacloprid were abandoned, as agreed with the Co-ordinator and the Project Officer, due to the inability to develop / formulate a gel with imidacloprid and potential novel synergists.

Musca domestica

Cypermethrin EC formulation and an Insect Growth Regulator, S-methoprene EC formulations (with, or without PBO and novel synergists) were studied and evaluated.

Test samples were supplied by UCSC and Babolna Bio. At the start of the studies, baseline toxicities were determined, and in both cases very low doses chosen.

Standard surface (glass) trials were chosen for cypermethrin studies, initially with susceptible and semi-resistant “A” and “B” populations, followed with the WHO strain. For IGR larvae studies, a special manure was selected in which the development (and/or the emergence inhibition) of *M. domestica* larvae were observed.

Following a number of trials, EN1-216, EN1-218 and EN1-126 were identified for further evaluation with a very low dose of Biopren (2.5 mL). Results showed equipotent synergism, all superseding Biopren 20 EC alone.

Beneficial insects

A study of possible adverse effects of the synergist/pesticide regime on beneficial insects was conducted. Bioassays with pesticides and the novel synergists were performed on *Apis mellifera* (honeybee) and *Bombus terrestris* (bumblebee). A preliminary evaluation on synergist toxicity was carried out using acute oral and contact assays including PBO for comparison. Overall, toxicity for the synergists alone was low except for EN 1-215 which was eliminated from further studies.

All the synergist bioassays were performed with EC formulations, as applicable for field conditions. Two pyrethroids (alpha-cypemethrin-FASTAC and tau-flavulinate-MAVRIK) were tested in combination with the synergists as well as two neonicotinoids: thiacloprid (CALYPSO) and imidacloprid (CONFIDOR). With the exception of imidacloprid, no synergism was observed.

WP5

The aim of WP5 was to evaluate the novel synergists chosen in WP4 (laboratory tests) in glasshouse and in semi-field conditions, to enable an informed decision based on conditions close to real applications. The details are provided as deliverable 5.1 and summarized below.

Based on the *in vivo* results obtained in the laboratory, the novel synergists used were EN1-126, EN1-213, EN1-215, EN1-216 and EN1-218.

EN1-215 was included on the basis of laboratory tests on *B. germanica*, even though the preliminary evaluation of the toxicity tests on pollinators was undesirable.

The novel synergists were evaluated on *Myzus persicae*, *Bemisia tabaci*, *Meligethes aeneus* and *Musca domestica.*

Myzus persicae

The proposed field trials in UK with aphids on oilseed rape was not carried out due to destruction of the trail area by cabbage stem flea beetles. Replacement trials were conducted in glasshouses during the winter 2013-2014. Results of synergism studies, including PBO for comparison, on oilseed rape infested with aphids at Debanham, Suffolk, UK, were negative. However, pymetrozine (Plenum), a feeding blocker for aphids, gave positive results. The hypothesis for the negative effect was attributed to the difficulty in hitting the target insects feeding on the undersurface of large leaves in a field situation. The importance of formulation plays a very important role in the success or failure of the trials and will need to be addressed in further developments.

Further trials on other insects were carried out in Turkey.

Results of tests conducted using *Aphis gossypii* on lettuce (in the absence of significant infestations of *M. persicae*) showed a high mortality produced by EN 1-218 in combination with thiamethoxam and cypermethrin when compared with the actives alone.

Bemisia tabaci

Preliminary trials set up in UK to test the concept of using synergists with insecticides to control pests, such as whiteflies in tomatoes, were unsuccessful. In the case of tomatoes, it was discovered during the course of raising plants for the proposed trial with whiteflies, that the glasshouse facilities to be used to carry out the trial would not/did not pass strict inspections by British government authorities for a non-indigenous licensable pest. Further work on whiteflies was therefore transferred to AITY in Turkey, where such restrictions and licenses were not required, as the pest was indigenous there.

A set of experiments against natural resistant populations of *Bemisia tabaci* were carried out.

Three different trials with synergist and pesticide combinations on *Bemisia tabaci* provided useful results. In these field (glasshouse) experiments the following synergists were used: EN1-218 (1-218), EN1-126 (1-126), EN1-215 (1-215), EN1-213 (1-213) and piperonyl butoxide (PBO), combined using split application (4 hours)

or a tank-mix with the following insecticides (commercial formulations): deltamethrin, cypermethrin, thiamethoxam and imidacloprid.

The crops were: pepper, eggplant and cucumber and a randomized block design was used in the experiments

The preliminary results did not differentiate between the novel synergists. Thereafter, synergists were combined with a reduced (2/3) dose of the insecticides. The insecticides used were limited to cypermethrin and thiamethoxam.

Table 6 shows that EN 1-218 gave positive results when used together with either insecticide. The efficacy of the insecticide alone and the efficacy of the insecticide plus EN1-218 were statistically different 6 and 10 days after application.

Table 6. Efficacy against *B. tabaci* larvae on cucumber. A reduced (2/3) dose of different insecticides was applied together with the synergists (tank-mix) (DAA: days after application).

Treatment	3 DAA Efficacy %	6 DAA Efficacy %	10 DAA Efficacy %
1-218 + Actara (thiamethoxam)	**26.87**	**63.53 a**	**72.37 a**
1-218 + Arrivo (cypermethrin)	**26.03**	**64.46 a**	**72.81 a**
1-126 + Actara (thiamethoxam)	**29.34**	**67.53 ab**	**61.41 ab**
1-126 + Arrivo (cypermethrin)	**29.36**	**67.67 ab**	**64.10 ab**
1-215 + Actara (thiamethoxam)	**21.25**	**51.40 b**	**52.02 b**
1-215 + Arrivo (cypermethrin)	**27.69**	**59.38 ab**	**65.42 ab**
1-213 + Actara (thiamethoxam)	**20.97**	**62.50 ab**	**58.87 ab**
1-213 + Arrivo (cypermethrin)	**24.55**	**58.92 ab**	**62.77 ab**
1-218	**10.73**	**12.80 c**	**5.70 c**
1-126	**11.23**	**20.43 c**	**9.87 c**
1-215	**6.88**	**13.87 c**	**6.99 c**
1-213	**10.09**	**12.61 c**	**9.36 c**
Actara (thiamethoxam)	**23.56**	**58.07 b**	**53.57 b**
Arrivo (cypermethrin)	**23.97**	**62.65 ab**	**59.47 ab**

The test was repeated with a lower (1/2) dose of thiamethoxam and cypermethrin in combination with EN1-218 and PBO as a comparison against adults (see Tables 13 and 14).

Meligethes aeneus

Trials were set up to test the efficacy of the novel synergists in comparison with PBO in combination with Fastac (alpha-cyperrmethrin) and Plenum (pymeytrozine) using pollen beetle on mustard, before flowering, when pollen beetle abundance was highest. .

The synergists used were EN1-126, EN1-215, EN1-213 ,EN1-218, EN1-216 and PBO as a comparison with the following treatments:-

a) Plenum 500 WG (0.15 kg/ha)
b) Fastac 100 EC (0.12 L/ha)
c) PBO 400 mL/ha
d) Novel synergists 400 mL/ha

Before treatment, the average number of pollen beetles per plant was calculated. Then the same calculation was made 1, 3, 7, and 11 days after the treatment. All these calculations were based on the numbers of pollen beetles on 100 plants within each plot.

On the basis of these calculations the efficacy of all combinations was established, according to the Henderson-Tilton's formula (Table 7)

Efficacy [%] = (1 - n in Co before treatment * n in T after treatment /

n in Co after treatment * n in T before treatment) * 100

Where n = average number of insect per one plant, T = treated , Co = control

Table 7. Efficacy of pesticides against pollen beetle. Control 1, 3, 7 and 11 days after the application (DAA: days after the application).

Treatment	1 DAA	3 DAA	7 DAA	11 DAA
2.Plenum500WG	65.00 %	40.10 %	53.86 %	5.25 %
3.Fastac 100EC	45.61 %	16.52 %	43.40 %	12.15 %
4.PBO	42.69 %	18.67 %	33.62 %	31.99 %
5.1-126	33.65 %	3.10 %	43.77 %	41.57 %
6.1-215	37.40 %	20.34 %	51.90 %	31.22 %
7.1-213	37.66 %	27.95 %	44.96 %	47.18 %
8.1-218	39.82 %	16.60 %	32.35 %	28.85 %
9.1-216	31.60 %	14.54 %	69.27 %	53.93 %
10.PBO+Fastac	**95.69 %**	**96.16 %**	**92.49 %**	**70.85 %**
11.1-126+Fastac	95.70 %	93.46 %	92.98 %	70.30 %
12.1-215+Fastac	**96.49 %**	**97.16 %**	**84.99 %**	**75.04 %**
13.1-213+Fastac	95.50 %	94.65 %	85.83 %	61.74 %
14.11-218+Fastac	**95.45 %**	**91.60 %**	**91.96 %**	**69.15 %**
15.1-216+Fastac	**93.81 %**	**92.79 %**	**74.94 %**	**74.75 %**

A reduction in the mean number of insects per plant was recorded particularly for 1 and 3 days after the application in all the treatments where insecticide + synergist were sprayed. The efficacy of all the products was high just after the application, but 3 DAA the insecticide + synergist mixtures maintained their efficacy. The highest synergistic ratio was observed 3 DAA (Table 8).

Table 8. Synergistic ratios calculated 1, 3, 7 and 11 days after the application of the products.

	synergistic ratio (I&S)/(I+S)			
Product	**1DAA**	**3DAA**	**7DAA**	**11DAA**
10 PBO (400 mL/ha) + Fastac (0.12 L/ha)	**1.08**	**2.73**	**1.20**	**1.61**
11 1-126 (400 mL/ha) + Fastac (0.12 L/ha)	**1.21**	**4.76**	**1.07**	**1.31**
12 1-215 (400 mL/ha) + Fastac (0.12 L/ha)	1.16	2.64	0.89	1.73
13 1-213 (400 mL/ha) + Fastac (0.12 L/ha)	1.15	2.13	0.97	1.04
14 1-218 (400 mL/ha) + Fastac (0.12 L/ha)	**1.12**	**2.77**	**1.21**	**1.69**
15 1-216 (400 mL/ha) + Fastac (0.12 L/ha)	1.22	2.99	0.67	1.13

Musca domestica

Further tests were performed using PBO and EN 1-216 adopting the same protocol as reported previously in WP4. In these tests, Cymina performance (efficacy) was poor (67.01% mortality), however this result had been achieved within almost 10 minutes and although after 40 minutes the result was 78 %, due to revival ended with 67%. It became quite clear that while the knock-down capacity is acceptable, the killing effect is not strong enough and does not reach the standard requirement of 90 % (but this may be due to be the fact that very low doses were used). Cymina + EN 1-216 EC acted extremely well (98.97 %), kept the speed of action and performed the result within 20 minutes.

The efficacy of PBO alone (6.06 g) was 87.63 % which is extremely high, but acted somewhat slowly at the beginning, with the killing effect speeding up after 90 minutes. PBO was used at two different application rates (3.03 and 1.01). 3.03 g PBO gave 71.43 %, but at the beginning of the trial PBO showed a very slow effect. 1.01 g PBO still gave 61.24 % with the same slow mode of action.

In order to find out whether any of the PBO 80 EC components had additional killing effects, Endura prepared a blank 80 EC formulation without PBO. The results showed no effect, therefore only PBO is considered responsible for the synergism**Errore. L'origine riferimento non è stata trovata.**.

These results clearly show that in the susceptible strain the results were excellent and have even surpassed the Cymina alone results. In the case of the less susceptible strain, the results were still good, considering the low concentration of the synergists. The overall conclusion of these studies is that the chosen synergists all have a certain killing effect and as such can be considered as an insecticide against the housefly.

A complete ANOVA test considering data from all the experiments was statistically significant. Mean mortality comparisons differentiated well between efficacy of insecticide alone and insecticide plus synergists. Efficacy of EN1-216 combined with Cymina was the highest and statistically different from all the remaining treatments. In both lines of study EN1-126, EN1-216 and EN1-218 performed above or close to PBO, but all performed well and have increased the efficacy of the insecticides.

Further studies however are necessary to establish the efficacy and mode of action in the case of resistant public health insects.

Beneficial insects

Tests were carried out to evaluate the toxicity of EN 1-126, EN 1-213, EN 1-215, EN 1-216 and EN 1-218 on beneficial insects (*A. mellifera* and *B. terrestris*) by themselves and in combination with two pyrethroids (alpha-cypermethrin (Fastac) and tau-fluvalinate (Mavrik)) and two neonicotinoids (imidacloprid (Confidor) and thiacloprid (Calypso)). On the basis of the results (Table 9), the potential synergists for agro applications had the following rating:

Table 9. Rating of novel synergists against honeybees and bumblebees when used in combination with considered insecticides.

	Mavrik		Fastac	
Synergist	honeybees	bumblebees	honeybees	bumblebees
EN 1 - 126 80 EC	+++	+++	+++	+++
EN 1 - 213 80 EC	+++	+++	++	+++
EN 1 - 215 80 EC	+++	+++	++	+++
EN 1 - 216 80 EC	+++	++	++	+++
EN 1 - 218 80 EC	+++	+++	+++	+++
	Calypso		Confidor	
Synergist	honeybees	bumblebees	honeybees	bumblebees
EN 1 - 126 80 EC	++	+++	++	+++
EN 1 - 213 80 EC	+	+++	+	+++
EN 1 - 215 80 EC	+	+++	+	+++
EN 1 - 216 80 EC	+	+++	+	+++
EN 1 - 218 80 EC	+	+++	+	+++

Where : +++ means suitable; ++ means indifferent; + means risk

WP6

The aim of WP6 was to evaluate the potential of synergist/insecticide applications to select for resistance compared with application of insecticide alone. A detailed report of the results of this WP is provided as deliverable 6.1. Therefore progress is provided in summary form only below.

Selection of a field population of *Bemisia tabaci* was carried out using alpha-cypermethrin with and without 100 ppm PBO and with thiacloprid with and without 100 ppm PBO. The selection was carried out until January 2015 corresponding to generation 8 of the whitefly population. The total number of generations under selection pressure was 6 generations. At this point final full dose-response bioassays were carried out to assess the susceptibility of the selected and unselected populations. In the case of *M. persicae* selections were carried out using the same treatment regimes as reported in PR1, these started on July 7th and selection pressure was applied every 2 weeks. A final bioassay was performed on November 26th, 2014.

In the case of both *M. persicae* and *B. tabaci* all four treatment regimes resulted in a significant increase in resistance (see Tables 10-12) with higher selection for resistance observed for thiacloprid than alpha-cypermethrin in both cases of insecticides treatment alone and insecticide treatment in combination with PBO.

Interestingly both thiacloprid+PBO and alpha-cypermethrin+PBO gave lower levels of resistance than when insecticide was used alone. For *M. persicae* this was statistically significant for both compounds but for *B. tabaci* this finding was only statistically significant for alpha-cypermethrin.

Taken together this finding suggests that when PBO is used in combination with insecticide it may slow the development of resistance, possibly by inhibiting the evolution of P450-mediated and/or esterase-mediated resistance.

Although this is a very interesting finding this experiment was conducted under controlled environment conditions (with a limited number of insects compared to a field scale scenario) and the study needs to be replicated in the field to confirm this.

Table 10. LC50 values and resistance ratios of the 8th generation of *B. tabaci* strains after different selection scenarios.

Selection	Bioassay treatment	LC_{50}	(95 % CL)	Slope ± SE	Resistance ratio
unselected	alpha-cyp.	3.72	(2.4-5.82)	1.244 0.117	-
	alpha-cyp. + PBO	3.44	(2-5.8)	1.181 0.149	-
alpha-cyp.	alpha-cyp.	103.1	(75.1-141)	1.392 0.112	28
	alpha-cyp. + PBO	64.9	(48.6-86.3)	1.36 0.101	19
alpha-cyp. + PBO	alpha-cyp.	39.8	(25.6-61.8)	1.225 0.127	11
	alpha-cyp. + PBO	24	(15.02-38.2)	1.336 0.16	6
unselected	thiacloprid	8.58	(5.93-12.2)	1.234 0.112	-
	thiacloprid+PBO	8.37	(5.84-11.9)	1.152 0.092	-
thiacloprid	thiacloprid	1466	(1250-1706)	2.367 0.162	171
	thiacloprid+PBO	718	(527-992)	1.866 0.183	86
thiacloprid+PBO	thiacloprid	1060	(746-1507)	2.014 0.245	124
	thiacloprid+PBO	1015	(810-1274)	1.872 0.143	118

Table 11. LD variations observed at the end of the selection experiment with *M. persicae* using Thiacloprid (Calypso - Ca) and Calypso + PBO (Ca+P).

Population	selected vs unselected		selected vs starting		unselected vs starting	
	Ca	Ca + P	Ca	Ca + P	Ca	Ca + P
LD_{50} ratio	**28.4**	**20.2**	**72.3**	**49.5**	**2.5**	**2. 4**
LD_{90} ratio	**0.6**	**14.0**	**0.5**	**0.8**	**2.3**	**1.7**

Table 12. LDs variations observed at the end of the selection experiment with M. persicae using alpha-cypermethrin (Fastac - F) and Fastac + PBO (F+P).

Population	selected vs unselected		selected vs starting		unselected vs starting	
	F	F+ P	F	F+ P	F	F+ P
LD_{50} ratio	**77.3**	**13.3**	**8.2**	**0.48**	**0.10**	**0.04**
LD_{90} ratio	**761.2**	**18.0**	**2.9**	**0.00**	**0.00**	**0.00**

We used RNAseq to compare global gene expression levels in selected/unselected *B. tabaci* and *M. persicae* populations with the RNAseq data received was of excellent quality.

As expected selection of both *B. tabaci* and *M. persicae* with thiacloprid and alpha-cypermethrin (both with and without PBO) resulted in measurable changes in gene expression. This included in genes with known roles in insecticide detoxification, such as cytochrome P450s, GSTs, CEs and ABC transporters.

Interestingly in the case of both insect species a much greater number of detoxification genes were upregulated in the strains selected with both insecticide and PBO. This is consistent with previous research which has shown that PBO induces the expression of both P450s and GSTs in *Drosophila melanogaster*.

In terms of candidate genes that have previously been shown to metabolise the insecticides used in our selection experiments, it was noteworthy that for *B. tabaci*, selection with thiacloprid (both with and without

PBO) resulted in a substantial increase in the expression of the P450 *CYP6CM1*. This finding is significant as this P450 has previously been shown to detoxify thiacloprid and therefore likely explains, at least in part, the resistance seen in the *B. tabaci* selected strains. In the *B. tabaci* cypermethrin selection experiment a strong candidate gene(s) that explained the resistance of the selected strains was not so apparent, particularly in the case of selection with cypermethrin alone. However, *CYP6CM1* was again upregulated after selection with cypermethrin+PBO although not at the same levels as selection with thiacloprid. At least three other P450s were overexpressed in this selected strain.

To our surprise very few candidate genes were identified that might explain the difference in the levels of resistance of the *B. tabaci* strains selected with insecticide alone and those selected with insecticide+PBO with a cuticular protein the only candidate gene overexpressed in the cypermethrin alone selected strain and no strong candidates overexpressed in the thiacloprid alone selected strain.

For *M. persicae* a much greater number of genes were differentially expressed and a large number of 'core' genes were consistently differentially expressed in all selected strains (5199 genes) compared to the non-selected strains. In terms of candidate genes that may explain the resistance phenotype of the selected strains a number of strong candidate genes were highly overexpressed in these strains. These included esterases matching FE4, which is known to have detoxification activity against pyrethroids, an ABC transporter which may play a role in phase III metabolism (excretion of phase I and II metabolites) and several P450s. Interestingly in the thiacloprid selected strains this included the P450 *CYP6CY3* which has been shown previously to detoxify neonicotinoid insecticides.

Analysis of *M. persicae* populations for target-site resistance mechanisms revealed that the frequency of all mutations actually decreased over the course of the experiment in all cases. This was a surprising finding and included the unselected population which also lost most of the target-site mutations (apart from R81T) over the course of the experiment, possible due to fitness penalties associated with these mutations which provided a selective advantage to a clone that did not carry these mutations. Analysis of target-site resistance in *B. tabaci* showed that the frequency of the L925I kdr mutation increased in frequency but only after selection with cypermethrin. This finding is logical and suggests, as expected, that selection with PBO does not avoid the selection of target-site resistance.

WP7

The aim of WP7 was to validate in the field or glasshouse the most effective novel synergist(s) and to check and estimate the scale up of the selected synergists. A detailed report of the results of this WP is provided as deliverable 7.1 and 7.2. Therefore, progress is provided in summary form only below.

Based on the results obtained in WP5 and on the chemical feasibility and cost, EN 1-216 and 1-218 were chosen as selected synergists. In particular, tests carried out with thiamethoxam and cypermethrin showed good results on adult *B. tabaci (*whitefly) at 2/3 and ½ of product field rate (Table 13).

Table 13. Efficacy of thiamethoxam with or without synergists against whiteflies on cucumber. 2015.

TREATMENTS	NIMPH				ADULT					
	Efficacy(%)									
	3 DAA		6 DAA		1 DAA		3 DAA		6 DAA	
1-216 (40ml/da)	37.24	a	37.42	c	21.41	e	13.54	d	26.38	c
1-216 + Actara(Thiomethoxam) (1/2 prod.rate)	46.62	a	83.43	a	44.71	cd	44.60	bc	35.27	bc
1-216+ Actara(Thiomethoxam) (2/3 prod.rate)	31.76	a	90.07	a	70.39	bc	83.64	a	76.23	ab
1-218 (40ml/da)	52.31	a	46.56	c	28.74	de	27.49	cd	25.96	c

1-218 + Actara(Thiomethoxam) (1/2 prod.rate)	52.37	a	84.54	a	89.84	ab	92.48	a	87.04	a
1-218+ Actara(Thiomethoxam) (2/3 prod.rate)	55.74	a	88.64	a	93.09	a	94.99	a	89.87	a
PBO (40ml/da)	67.91	a	40.63	c	29.46	de	13.08	d	15.91	d
PBO+ Actara(Thiomethoxam) (1/2 prod.rate)	65.69	a	74.52	b	85.47	ab	90.66	a	89.26	a
PBO+ Actara(Thiomethoxam) (2/3 prod.rate)	60.91	a	82.78	a	89.70	ab	91.95	a	89.76	a
Actara(Thiomethoxam) (1/2 prod.rate)	67.70	a	68.59	b	56.24	c	52.61	bc	42.49	b
Actara(Thiomethoxam) (2/3 prod.rate)	63.19	a	76.85	ab	68.59	bc	71.06	ab	59.61	ab

Table 14. Efficacy of cypermethrin with or without synergists against whiteflies on cucumber. 2015.

TREATMENTS	NIMPH				ADULT					
	Efficacy(%)									
	3 DAA		6 DAA		1 DAA		3 DAA		6 DAA	
1-216 (40ml/da)	37.24	a	37.42	d	21.41	d	13.54	b	26.38	d
1-216+Arrivo(Cypermethrin) (1/2 prod.rate)	32.37	a	86.33	a	86.72	a	90.44	a	79.65	bc
1-216+Arrivo(Cypermethrin) (2/3 prod. rate)	59.24	a	91.31	a	87.94	a	90.29	a	81.89	b
1-218 (40ml/da)	52.31	a	46.56	c	28.74	cd	27.49	b	25.96	d
1-218+Arrivo(Cypermethrin) (1/2 prod.rate)	60.44	a	78.69	ab	92.75	a	92.48	a	90.35	a
1-218+Arrivo(Cypermethrin) (2/3 prod.rate)	63.87	a	90.29	a	93.78	a	94.06	a	90.94	a
PBO (40ml/da)	67.91	a	40.63	c	29.46	cd	13.08	b	15.91	d
PBO+Arrivo(Cypermethrin) (1/2 prod.rate)	56.60	a	76.72	b	95.31	a	93.95	a	89.86	a
PBO+ Arrivo(Cypermethrin) (2/3 prod.rate)	61.27	a	87.96	a	95.81	a	95.14	a	89.33	a
Arrivo(Cypermethrin) (1/2 prod.rate)	39.71	a	72.34	bc	48.42	bc	80.37	a	59.43	c
Arrivo(Cypermethrin) (2/3 prod.rate)	64.19	a	78.16	b	53.19	b	82.78	a	67.10	bc

EN1-218 was the most effective synergist in combination with the actives at 2/3 and 1/2 of the product label rate.

The same synergistic efficacy was observed with *A. gossypii* on lettuce where EN 1-218 had the highest synergistic effect.

The mortality produced by EN1-216 and EN1-218 on *A. mellifera* was checked in the presence of a neonicotinoid (thiacloprid-Calypso) and a pyrethroid (tau fluvalinate-Mavrik). No significant differences were observed between the tested synergists whilst a difference was observed between Calypso and Mavrik. In particular, no differences were observed between the active ingredient when applied alone and in combination with the novel synergists.

An economic evaluation regarding the possible industrial cost of EN1-216 and EN1-218 was carried out and the industrial cost of the selected synergist is similar to, or slightly less, than the cost of PBO.

- **The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results** (not exceeding 10 pages).

WP2 demonstrated that the structure of the analogue could be predicted from SAR studies to give higher inhibition of the metabolic enzyme(s). Thus, the insect's defence to insecticides could be minimised to greatly increase the effect of insecticide application. Clearly this promotes the possibility of controlling even highly insecticide-resistant insects with a field-rate, or lower, application of insecticide. Such a scenario has the potential to decrease the application rates of insecticides with the concomitant environmental benefits.

Data collected during WP4 activities shows that novel structures can positively synergize neonicotinoids and pyrethroids against economically important agricultural and household pests. Their effect *in vivo* is similar and in several cases better than that of PBO. But the optimal combination of insecticide / synergist is usually different according to pest species.

WP3 confirmed the patentability of some novel structures proposed by SAR studies and *in silico* modelling, as well as their chemical feasibility. Patentability infers a positive evaluation of the costs of the novel synergists based on the industrial steps involved in their synthesis. The cost/performance ratio was taken as a parameter to consider in the subsequent *in vivo* evaluation of the novel synergists, where only an exceptionally good activity could justify the development of the novel synergist if the synthesis pathway represented a high level of complexity.

A part of WP 4 focused on public health pests and two species were chosen, *Musca domestica* and *Blattella germanica*. It is well known to the industry participants and to the members of the pest control industry that due to the Biocidal Product Regulation a major review of the active substances has been undergoing since 1988. While at the beginning more than 1700 actives were notified, by today less than 700 actives are still in the system, all the others were banned from use. This fact radically cuts not only the availability of insecticides, but their efficacy, and furthermore contributes in developing resistance more rapidly. In these experiments the EcoSyn project aimed to develop eco-friendly synergist(s) with the purpose of increasing the efficacy of traditional insecticides and possibly decrease their doses.
In a great number of public health pests, resistance is being observed. Fleas, bed bugs, cockroaches, fly etc. – which pose serious health threats - all have their insecticide defence mechanism(s). Under such circumstances novel synergists may contribute by making the available insecticides more capable and efficacious, or by slowing the development of resistance.
In many ways WP 4 demonstrated that a few of the chosen novel synergists might possess these capabilities. Whether the use of the novel synergists, in combination with insecticides, slows the development of resistance remains to be confirmed by further studies.

In WP5 the novel synergists identified from the trials carried out in WP4, were evaluated in more detail in glasshouse and semi-field conditions which further narrowed the choice of effective candidates. That evaluation, together with the possible industrial costs of the potential synergists, and the cost and availability of the starting materials, resulted in a more restricted choice of potential synergists. On that basis, only two synergists were chosen to be validated in WP7.

In WP6 results demonstrated that in the case of two economically important insect pests (*M. persicae* and *B. tabaci*) selection with two insecticides (thiacloprid and alpha-cypermethrin) in combination with the synergist PBO gave lower levels of resistance than when insecticide was used alone. This finding suggests that when PBO is used in combination with insecticide it may slow the development of resistance, possibly by inhibiting the evolution of P450-mediated and/or esterase-mediated resistance. This is a very interesting finding with significant potential impact. If this can be replicated at a field scale it could form the basis of a resistance

management strategy to slow the development of resistance. Because there are limited novel insecticides/modes-of action, strategies and tools to prevent or slow the development of resistance are urgently required. Therefore, additional funding should be sought to confirm this finding as a matter of some urgency.

In WP7 the validation carried out in large glasshouses identified two novel synergists, the costs of which were comparable to, or better than, the commercial synergist PBO. IP considerations resulted in three patent applications being filed, enabling the possibility of commercial exploitation.

- **The address of the project public website, if applicable as well as relevant contact details**

EcoSyn project website - http://ecosyn.eu/

All the project's open access publications and dissemination materials are available on the following link: http://ecosyn.eu/index.php?lingua=uk&pagina=4100

Contact details:
Endura S.p.a.
Dr Valerio Borzatta
+39 051 5281709
vborzatta@endura.it

http://ecosyn.eu/index.php?lingua=uk&pagina=4103

4.2 Use and dissemination of foreground

A plan for use and dissemination of foreground (including socio-economic impact and target groups for the results of the research) shall be established at the end of the project. It should, where appropriate, be an update of the initial plan in Annex I for use and dissemination of foreground and be consistent with the report on societal implications on the use and dissemination of foreground (section 4.3 – H).

The plan should consist of:

- Section A

  This section should describe the dissemination measures, including any scientific publications relating to foreground. **Its content will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

- Section B

  This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential **will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[2] (if available)	Is/Will open access[3] provided to this publication?
1	**The interactions between piperonyl butoxide and analogues with the metabolic enzymes FE4 and CYP6CY3 of the green peach aphid Myzus persicae (Hemiptera: Aphididae).**	Panini, Michela; Tozzi, Francesco; Bass, Chris; Zimmer, Christoph T.; Field, Linda; Borzatta, Valerio; Mazzoni, Emanuele; Moores, Graham	Pest Management Science	submitted in December 2015 – manuscript no. PM-15-0798	John Wiley & Sons, Inc.		2016 (under review)			no
2	**Vcely v současné ochrane rostlin [Honeybees and recent plant protection]**	Titera, D.	Rostlinolekar	*ISSN* (Print): 1211-3565	Ceska spolecnost rostlinolekarska	Praha (Czech Republic)	2016 (under review)	1200 words	n.a.	no

[2] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[3] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	**Type of activities[4]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[5]**	**Size of audience**	**Countries addressed**
1	Press release	Universita Cattolica Del Sacro Cuore	Sinergizzanti , le nuove sostanze per ridurre uso ed effetti degli insetticidi	October 2013	Piacenza (Italy)	Research, Industry	-	IT
2	Press release (web site)	Universita Cattolica Del Sacro Cuore	Ecosyn, sinergizzanti per ridurre l'effetto nocivo degli agrofarmaci	October 2013	www.agrinotizie.it	Industry	-	IT
3	Press release	Endura	EU funds insecticide synergist research	November 2013	www.agra-net.com	industry	-	worldwide
4	Press releases	Rothamsted	Reducing the impact of pesticides on beneficial insects	November 2013	UK	Scientific community		UK
5	Press releases	Babolna	Synergists sought to reduce insecticide resistance	November 2013	"Pest Magazine"	Industry		International
6	Presentation	Rothamsted	AgriFood Advanced Training Partnership: Current Research in Crop Protection	February 2014	Rothamsted	Industry	15	UK
7	Oral presentation to a scientific event	Universita Cattolica Del Sacro Cuore	Formulation Resistance Challenges	February 2014	Berlin (Germany)	Scientific community (higher education, Research) - Industry	150	Europe
8	Presentation	Universita Cattolica Del Sacro Cuore	Presentation of project aims and implications and non-confidential results to technicians during regional meeting dealing with insecticide resistance management	February 2014	Apulian Region, Italy	Scientific community, technicians, Regulatory officers	100	Italy
9	Conference (+ poster)	Rothamsted	Emerging science and technologies in crop	March 2014	Agri - innovation 2014 Imperial	Scientific Community	250	UK

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

			research ECOSYN : A project supported by the European Union for the development of novel,eco-friendly synergists		College London, UK			
10	Oral presentation	Universita Cattolica Del Sacro Cuore	EcoSyn :un progetto supportato dalla Comunità Europea per lo sviluppo di nuove molecole sinergizzanti	March 2014	Chianciano Terme, Italy	Scientific community (higher education, Research)	150	Italy
11	Conference	Rothamsted	European Congress of Entomology	August 2014	University of York, UK	Scientific Community	500	Europe
12	Conference (poster)	Rothamsted	IUPAC International Congress of Pesticide Chemistry	August 2014	Conference facility, San Francisco, US	Scientific Community	>1000	International
13	Conference (poster)	Universita Cattolica Del Sacro Cuore	ECOSYN : A project supported by the European Union for the development of novel,eco-friendly synergists	August 2014	10th Euroean Congress of Entomology Heslington, York, UK	Scientific community (higher education, Research)	300	European Countries
14	Press releases	ENDURA	EcoSyn insecticide synergist research progress	August 2014	AGROW	Industry, scientific community		Worldwide
15	Press releases	ENDURA	Update on EcoSyn project	September 2014	Chemistry Today	Industry		European Countries
16	Press releases	Universita Cattolica Del Sacro Cuore	Con Ecosyn pesticidi più ecocompatibili	October 2014	Cattolica News	Scientific community (higher education, Research)		Italy
17	Conference	DCP	Crop Protection in Southern Britain	November 2014	Peterborough, UK	Industry, scientific community		UK
18	Press releases	Babolna	Ecosyn project	November 2014	Hungarian Pest Control Association	Industry, research		Hungarian
19	Poster	Vyzkumny Ustav Vcelarsky Sro	ECOSYN: Projekt Evropské unie podporuje vyvoj novych k prirode setrnych synergistu v	November 2014	Pardubice /Czech Republich	Scientific community (higher education, Research)	200	Czech participants

			zemedelstvi					
20	Presentation	Università Cattolica Del Sacro Cuore	Presentation of project aims and implications and non-confidential results to technicians and Regulatory authorities	February 2015	Servizio Fitosanitario – Regione Emilia Romagna, Bologna, Italy	Scientific community, technicians, Regulatory officers	50	IT
21	Presentation	Università Cattolica Del Sacro Cuore	Presentation of project aims and implications and non-confidential results to technicians during regional meeting dealing with insecticide resistance management	March 2015	Basilicata Region, Italy	Scientific community, technicians, Regulatory officers	100	Italy
22	Conference	Rothamsted	Agri Innovation 2015: Emerging Science and Technologies in Crop Research	April 2015	London, UK	Scientific Community	250	UK/ Europe
23	Workshop	Ankara Ileri Teknoloji Yatirimlari Anonim Sirketi	Insect resistance management and synergism	April 2015	Ankara (Turkey)	Ministry of Agriculture - Commission for pesticide registration Scientific community	20	Turkey
24	Presentation	AgChem	Presentation to regulatory authorities in the UK (HSE) to outline the findings and the potential benefits of the use of EcoSyn synergists	April 2015	Norwich (UK)	Regulatory Authority	5	UK
25	Oral presentation + poster	Università Cattolica Del Sacro Cuore	Ecofriendly synergists for insecticide formulations (EcoSyn)	July 2015	London (UK) SCI'Agriscience Young Researchers Event	Scientific community (higher education, Research)	100	European Countries
26	Workshop – professional course	Vyzkumny Ustav VcelarskY Sro	A professional course for beekepers focused on EcoSyn findings	July 2015	Bee Research Institute Dol	Beekeepers	68	Czech Republic
27	Workshop	Ankara Ileri Teknoloji Yatirimlari Anonim Sirketi	Meeting on the project aims and implications and to disclose non-	July 2015	Tarsus (Mersin – Turkey)	Industry, agriculture association, agronomists	20	Turkey

			confidential results to farmers and agronomists of the Chambers of Agriculture					
28	Article	Rothamsted	Insecticide Boost	September 2015	C&I Magazine Issue 9	Industry	N/A	International
29	Posters	ENDURA ROTHAMSTED + ApresLab DCP UCSC BABOLNA AITY	Ecofriendly synergists for insecticide formulations (EcoSyn The following posters were presented: a) Ecofriendly synergists for insecticide formulations (EcoSyn): in vitro characterisation of interactions with detoxifying enzymes (with Apreslabs); b) Ecofriendly synergists for insecticide formulations (EcoSyn): in vivo evaluation of novel synergists against resistant pests; c) "Ecofriendly synergists for insecticide formulations (EcoSyn): in vitro and in vivo evaluation of novel synergists against Musca domestica" (with Babolna Bio); d) Ecofriendly synergists for insecticide formulations (EcoSyn): evaluating the potential of synergist/insecticide applications to select for resistance compared with application of insecticide alone (With Rothamsted)	September 2015	Rothamsted Research, Harpenden, Hertfordshire, UK – Resistance 2015	Scientific community (higher education, Research) - Industry		EU
30	Workshop	Rothamsted	Ecofriendly synergists for insecticide formulations (EcoSyn)	September 2015	Rothamsted Research, UK	Scientific Community/Industry	300	EU
31	Presentation	Università Cattolica Del Sacro Cuore	Presentation about Ecosyn project results with the title "Characterisation of interactions between novel inhibitors and metabolic enzymes in insect pest"	November 2015	6th annual meeting of the European PhD Network in "Insect Science", Florence	Scientific Community	70	EU

Section B (Confidential[6])
Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.
The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[7]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	Yes	08/03/2017	EP application n.15184405.7	Substituted methylenedioxybenzyl compounds and their use as synergists	Endura S.p.A. Babolna Bioenvironmental Centre Ltd. AgChem Access Ltd.
Patent	Yes	08/03/2017	EP application n.15184406.7	Substituted 2,3-dihydrobenzofuran compounds and their use as synergists	Endura S.p.A. Babolna Bioenvironmental Centre Ltd. AgChem Access Ltd.
Patent	Yes		Italian application n.102015000050013	Composti di diidrobenzofurano sostituiti e loro uso come sinergici	Endura S.p.A. Babolna Bioenvironmental Centre Ltd. AgChem Access Ltd.

[6] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[7] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2
Please complete the table hereafter:

Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
N/A								

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

- Its purpose
- How the foreground might be exploited, when and by whom
- IPR exploitable measures taken or intended
- Further research necessary, if any
- Potential/expected impact (quantify where possible)

The exploitation terms of the EcoSyn foreground are defined in the Consortium Agreement, agreed and signed by all project beneficiaries. The CA, art 8.2 Foreground, clarifies ownership of all generated foreground and terms which apply to the patentable foreground and filed patents applications. In particular the ownership of the patent applications was share among the attendants SMEs in accordance with the Consortium agreement and the related Side Agreements.

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.
[9] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

4.3 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	605740
Title of Project:	EcoSyn - Ecofriendly synergists for insecticide formulations
Name and Title of Coordinator:	Dr. Valerio Borzatta, Endura

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	No
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	No
• Did the project involve Human genetic material?	No
• Did the project involve Human biological samples?	No
• Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	Yes (insects)
• Were those animals transgenic small laboratory animals?	No
• Were those animals transgenic farm animals?	No
• Were those animals cloned farm animals?	No

• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	**No**
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	**No**
DUAL USE	
• Research having direct military use	0 Yes x No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator		1
Work package leaders	2	10
Experienced researchers (i.e. PhD holders)	3	8
PhD Students	1	0
Other	4	3

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	
Of which, indicate the number of men:	1

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

⊙ No
○

6. Which of the following actions did you carry out and how effective were they?

	Not at all effective				Very effective
❑ Design and implement an equal opportunity policy	○	○	⊙	○	○
❑ Set targets to achieve a gender balance in the workforce	○	○	⊙	○	○
❑ Organise conferences and workshops on gender	⊙	○	○	○	○
❑ Actions to improve work-life balance	⊙	○	○	○	○

○ Other:

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

⊙ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

⊙ Yes-please specify

Presentation about Ecosyn project results with the title "Characterisation of interactions between novel inhibitors and metabolic enzymes in insect pest" at the 6th annual meeting of the European PhD Network in "Insect Science", Florence, 11th – 13th November 2015 (Università Cattolica del Sacro Cuore with ApresLabs)

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○

⊙ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

⊙ Main discipline[10]: Entomology, Organic Chemistry, Biochemistry

⊙ Associated discipline[10]: Chemical Engineering, Analytical chemistry

○ Associated discipline[10]: Agronomy

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*

⊙ Yes (beekeepers, agronomists)

○ No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

[10] Insert number from list below (Frascati Manual).

- ○ No
- ⊙ Yes- in determining what research should be performed
- ○ Yes - in implementing the research
- ⊙ Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○	Yes
	⊙	No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ⊙ Yes- in framing the research agenda *(Regulatory Authorities)*
- ○ Yes - in implementing the research agenda
- ⊙ Yes, in communicating /disseminating / using the results of the project *(Regulatory Authorities)*

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ⊙ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

✓ Agriculture	Energy	Human rights
Audiovisual and Media	Enlargement	Information Society
Budget	Enterprise	Institutional affairs
Competition	✓ Environment	Internal Market
Consumers	External Relations	Justice, freedom and security
Culture	External Trade	✓ Public Health
Customs	Fisheries and Maritime Affairs	Regional Policy
Development Economic and Monetary Affairs	Food Safety	Research and Innovation
Education, Training, Youth	Foreign and Security Policy	Space
Employment and Social Affairs	Fraud	Taxation
	Humanitarian aid	Transport

13c If Yes, at which level?

- ⊙ Local / regional levels
- ⊙ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**In progress** (2 submitted, 2 in preparation)
To how many of these is open access[11] provided?	
How many of these are published in open access journals?	**All**
How many of these are published in open repositories?	**0**
To how many of these is open access not provided?	**N/A**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[12]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**3**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
■	Difficult to estimate / not possible to quantify		

[11] Open Access is defined as free of charge access for anyone via Internet.
[12] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:* 2
Difficult to estimate / not possible to quantify	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes ⊙ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes ⊙ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

■	Press Release		■	Coverage in specialist press	
❑	■	Coverage in general (non-specialist) press			
❑	❑				
❑	❑				
■	■	Website for the general public / internet			
■	■	Event targeting general public (festival, conference, exhibition, science café)			

23 In which languages are the information products for the general public produced?

■	Language of the coordinator	■	English
■	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]