

BIOCEBO/BIO

DOCUMENT M-CP, Section 6

EFFICACY DATA

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CP 6 EFFICACY DATA

CP 6.1 Preliminary range-finding tests - Minimum effective dose tests

Not available. Plant protection products containing hydrolyzed proteins acting as attractant has been present in the market for more than 10 years. Similar registered products were using the same range when BIOCEBO entered into the market.

CP 6.2 Efficacy tests

Six trials were carried on in Spain and Italy in 2011 and 2012 on peach, clementine and olive trees to check the efficacy of BIOCEBO and FLYRAL against *Ceratitis capitata* and *Bractocera oleae* pests.

BIOCEBO and FLYRAL are both products property of BIOIBERICA, which is the company manufacturing both pest protection products. Both products contain Hydrolysed proteins as its main and only ingredient, but the difference between both of them is the concentration in hydrolysed proteins (BIOCEBO contains 30% w/v of Hydrolysed proteins and FLYRAL 36% w/v).

Different applications (2-3) were done in each trial during the fruit maturing process and tested products were compared with a standard and an untreated control.

Results showed that both products reported to be a very good pest control, no problem was observed during handling of the test materials and no symptoms of phytotoxicity were observed.

The field trials summarized in this dossier are the following:

Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
DEVREG CONSULTA SLU Les Franqueses, 59 baixos E-25600 Balaguer, Lleida Spain	2013	Efficacy and selectivity of Flyral® and Biocebo® when applied as bait application together with conventional insecticide over peach/nectarine for <i>Ceratitis capitata</i> control. Spain 2012. Report number: 2012023 Unpublished	Y	BIOIBÉRICA, S.A.

MÉTODOS SERVICIOS AGRÍCOLAS S.L. C/ El Reguero s/n 30559 Abarán Murcia Spain	2011	FIELD TRIAL REPORT Testing the efficacy of the use of hydrolyzed protein on insecticide applications to the control of <i>Ceratitis capitata</i> on citrus. Report number: 11_bio.i01 Unpublished	Y	BIOIBÉRICA, S.A.
BIOFARM s.r.l – Caserta ViaMazzini Vico VI, n.1 81047 – Macerata Campania (CE) Italy	2013	Study: FLYRAL and BIOCEBO Efficacy and selectivity and evaluation of Flyral (Hydrolyzed protein 36%) and Biocebo (Hydrolyzed protein 30%) for mediterranean fruit fly or medfly (<i>Ceratitis Capitata</i> Certca) control on peach in Campania region (Italy). Report number: PC12 BBR 33 Unpublished	Y	BIOIBÉRICA S.A.
DEVREG CONSULTA SLU Les Franqueses, 59 baixos E-25600 Balaguer, Lleida Spain	2013	Efficacy and selectivity of different protein formulation together with conventional insecticide against <i>Dacus oleae</i> . Report number: 2012024 T1 Unpublished	Y	BIOIBÉRICA, S.A.
MÉTODOS SERVICIOS AGRÍCOLAS S.L. C/ El Reguero s/n 30559 Abarán Murcia Spain	2012	FIELD TRIAL REPORT Testing the efficacy of the use of hydrolyzed protein on insecticide applications to the control of <i>Bactrocera oleae</i> on olives. Report number: 11_bio.i02 Unpublished	Y	BIOIBÉRICA, S.A.
BIOFARM s.r.l – Caserta ViaMazzini Vico VI, n.1 81047 – Macerata Campania (CE) Italy	2013	Study: FLYRAL and BIOCEBO Efficacy and selectivity and evaluation of Flyral (Hydrolyzed protein 36%) and Biocebo (Hydrolyzed protein 30%) for olive fruit fly (<i>Bactrocera oleae</i> or <i>Dacus oleae</i> Dacuol) control on olive in Campania region (Italy). Report number: PC12 BBR 34 Unpublished	Y	BIOIBÉRICA, S.A.

A summary of each efficacy trial is provided in the following pages.

CP 6.3 Effects on yield and quality

Efficacy and selectivity of Flyral® and Biocebo® when applied as bait application together with conventional insecticide over peach/nectarine for *Ceratitis capitata* control.

Report number: 2012023

An efficacy trial was performed in stone fruits orchards to evaluate the efficacy of the test products Biocebo® and Flyral®, applied together with conventional insecticide as bait spot application, for the control of *Ceratitis capitata*.

The application of these products was compared to another equivalent product (Protsar), which was applied with the same methodology with untreated and with conventional insecticide.

Trial site was located in a typical peach/nectarine growing area in Fraga, Huesca, and the site was selected according the historical on *Ceratitis capitata* attack.

Two applications were conducted during one month before harvest as conventional practice in the area.

The following results were obtained:

- All treated plots achieve significantly better control levels compared with untreated plots.
- Test item Biocebo® applied together with conventional insecticide as bait spot application does not differ significantly compared with efficacy reached by conventional insecticide application for *Ceratitis capitata* control when fruit damage is observed.
- Treatment number 5, (Flyral®) efficacy level when fruit damage is observed, improves efficacy level reached by conventional insecticide application.

This efficacy levels when fruit damage has been assessed, have been produced with low pest pressure.

When adult monitoring captures have been assessed, significant differences between treated and untreated plots have been detected.

No phytotoxicity effects were detected.

Testing the efficacy of the use of hydrolyzed protein on insecticide applications to the control of *Ceratitis capitata* on citrus.

Report number: 11_bio.i01

A plot of mandarin variety Okitsu was selected in Pobla de Vallbona (Valencia), elemental plots were 800 m². Karate Zeon (bait application method) was sprayed in a mixture with the hydrolyzed proteins, applied in patch form at 80 l/ha with a backpack manual sprayer for three times. Spintor Cebo (bait application method) was sprayed in patch at 10 l/ha with a backpack manual sprayer for three times.

Two assessments were carried out at 7 DA-C and 14 DA-C for the percentage of damaged fruits on tree. 5 branches were marked per elemental plot, in the orientations north, south, east and west. In addition, we marked a branch in the south-east orientation, in total 5 branches. The counts of damaged fruits were done in these marked branches. This count method was the one applied by the Service of Plant Health, and we perform it according to his indications. 400 fruits were evaluated in these 5 marked branches, counting the damaged fruits per elementary plot.

At 19-10-2011, 400 fruits per plot (100 fruits per subsample) were harvested, and after 10 days in storage (24 DA-C), the percentage of damaged fruits were also assessed.

We obtained the percentage of damaged fruits with the number of damaged fruits divided by total fruits per plot (No. Damaged fruits x 100 / Total fruits).

Three assessments were carried out at 7 DA-A, 7 DA-B and 7 DA-C for number of *Ceratitis capitata* captures in traps. 1 trap was placed in the south face of the tree, at about 1.5 m high, following the habitual practice of placement of traps for capture of *Ceratitis capitata*. TRIPACK MFL (food attractant) and VAPONA (Dichlorvos insecticide) were used in monitoring traps.

Trees were also checked for possible symptoms of phytotoxicity at 7 DA-A, 7 DA-B and 7 DA-C. Abbott efficacy was calculated. The products applied were:

Trt No.	Type	Treatment Name	Form Conc	Form Unit	Form Type	Description	Rate	Rate Unit	Other Rate	Other Rate Unit	Appl Code
1	CHK	Untreated									
2	INSE	Spintor Cebo	0,024	%	CB	Spinosad	1,5	L/HA	0,36	g A/ha	ABC
3	INSE	Karate Zeon	10	%	CS	Lambda cihalotrim	125	mL/100 L	12,5	g A/100 L	ABC
	INSE	Biocebo	30	%	SL	Hydrolyzed protein	1,5	% W/V	450	g A/100 L	ABC
4	INSE	Karate Zeon	10	%	CS	Lambda cihalotrim	125	mL/100 L	12,5	g A/100 L	ABC
	INSE	Flyral	36	%	SL	Hydrolyzed protein	1,25	% W/V	450	g A/100 L	ABC
5	INSE	Karate Zeon	10	%	CS	Lambda cihalotrim	125	mL/100 L	12,5	g A/100 L	ABC
	INSE	Nulure	30	%	SL	Hydrolyzed protein	1,25	% W/V	375	g A/100 L	ABC

Results summarized as follows:

- Percentage damaged fruits: Statistically significant differences were observed between untreated and treated plots, but no between treated plots. The percentage of fruits damaged on untreated plot reached 13.5% at 7 DA-C, increased to 32% at 24 DA-C. Karate Zeon was sprayed in mixture with BIOCEBO or FLYRAL, applied in patch, and achieved excellent efficacies, similar to Karate Zeon when sprayed in mixture with NULURE, applied in patch, and to SPINTOR CEBO.
- Number of *Ceratitis capitata* captures: Statistically significant differences were observed between untreated and treated plots, but no between treated plots. The number of captures on untreated plot reached 13.5 at 7 DA-A, increased to 59% at 7 DA-B. Karate Zeon when sprayed in mixture with BIOCEBO or FLYRAL, applied in patch, achieved excellent efficacies, similar to Karate Zeon when sprayed in mixture with LUNURE applied in patch, and to SPINTOR CEBO.
- Karate Zeon was sprayed in mixture with an hydrolyzed protein applied in patch significantly decreased the damage on fruits and number of *Ceratitis capitata* compared to untreated control.
- No problems were detected when handling the experimental products.
- No symptoms of phytotoxicity were detected.

Efficacy and selectivity and evaluation of Flyral (Hydrolyzed protein 36%) and Biocebo (Hydrolyzed protein 30%) for mediterranean fruit fly or medfly (*Ceratitis Capitata* CERTCA) control on peach in Campania region (Italy).

Report number: PC12 BBR 33

The trial was carried out on Peach [*Prunus persica* (L.) Batsch var. Guglielmina], on field-grown, in Southern Italy (Campania), locality Vairano Patenora (CE), according to an experimental design with randomized blocks, side-by-side, length-wise layout and 4 replications.

One untreated plot is served as control, Karate Zeon 1.5 is authorized on Peach; Flyral and Biocebo are not authorized on Peach, even if their active substance was already authorized on Peach.

Nu Bait, based on Hydrolyzed protein, is authorized on peach.

No artificial infestation was done and natural infestation of Mediterranean fruit fly or Medfly (*Ceratitis capitata*) was naturally present.

In the trial period infection of not target infestation was observed: *Myzus persicae*.

Three applications were carried out at the phenological growth stage:

- Fruit about 90% of final size - Beginning of fruit colouring. (BBCH 79-81. Application: 31/07/2012)
- Beginning of fruit colouring - Colouring advanced (BBCH 81-85. Application: 10/08/2012)
- Colouring advanced - Fruit ripe for picking. (BBCH 85-87. Application: 20/08/2012)

According to the special authorization and the study plan requirements the applications on thesis 3,4 and 5 were made on one side of each row.

Summarized conclusions:

- Karate Zeon 1.5 at rate 0.125 L/ha and Biocebo at rate 1.5 L/ha ensured good protection of Peach in presence of very favourable conditions for natural infestation.
- Karate Zeon 1.5 at rate 0.125 L/ha and Flyral at rate 1.25 L/ha ensured good protection of Peach in presence of very favourable conditions for natural infestation.
- All the products have effectively contained the infestation.

The test substances were dispersed in water without any problem and solubility was perfect. The water used as carrier was tap water taken from the farm. No problem was observed during handling of the test materials. The weather conditions in field were favourable for the development of the infection.

Efficacy and selectivity of different protein formulation together with conventional insecticide against *Dacus oleae*.

Report number: 2012024 T1

An efficacy trial was conducted in olives orchards to evaluate the efficacy of test materials Biocebo® and Flyral®, applied together with conventional insecticide as bait spot application, for *Dacus oleae* control, in comparison with other equivalent product as Protsar® applied with same methodology with untreated and with conventional insecticide and program.

Trial site was located in a typical olive growing area in Mont Roig del Camp, Tarragona, and the site was selected according the historical on *Dacus oleae* attack.

Two applications were conducted during one month before harvest as conventional practice in the area.

The following results were obtained:

- All treated plots achieved significantly better control levels compared with untreated plots.
- Test items Biocebo® and Flyral® applied together with conventional insecticide as bait spot application does not differ significantly compared with efficacy reached by conventional insecticide application for *Dacus oleae* control when fruit damage is observed.
- Treatment number 3 with Protsar® efficacy level when fruit damage is observed, show significant differences compared with untreated plots (significantly higher) and with the other treatments (significantly lower).
- Treatment number 4 Biocebo® applied together with conventional insecticide as bait spot application achieve level of *Dacus oleae* adult captures significantly lower compared with captures level from untreated plot. No significant differences are observed between the rest of treated plots and untreated plot.
- This efficacy levels when fruit damage have been assessed, have been produced with high pest pressure. However, this high pressure does not allow us to show significant differences between treated plots.
- No phytotoxicity effects were detected.

Testing the efficacy of the use of hydrolyzed protein on insecticide applications to the control of *Bactrocera oleae* on olives.

Report number: 11_bio.i02

A plot of olive variety Cuquillo was selected in Moratalla (Murcia). Elemental plots were 2000 m². Karate Zeon (bait application method) was sprayed in a mixture with the hydrolyzed proteins, applied in patch form at 80 l/ha with a backpack motorized sprayer for three times. Spintor Cebo (bait application method) was sprayed in patch at 10 l/ha with a backpack motorized sprayer for three times.

Two assessments were carried out at 8 DA-B and 7 DA-C for the percentage of damaged fruits on tree. 5 branches were marked per elemental plot, in the orientations north, south, east and west. In addition, we marked a branch in the south-east orientation, in total 5 branches. The counts of damaged fruits were done in these marked branches. 800 fruits were evaluated in these 5 marked branches, counting the damaged fruits per elementary plot.

At 09-11-2011, 800 fruits per plot (200 fruits per subsample) were harvested, and after 7 days in storage (14 DA- C), the percentage of damaged fruits were assessed also.

At 16-11-2011, the number of fallen fruits on soil due to *Bactrocera oleae* was assessed on field.

We obtained the percentage of damaged fruits with the number of damaged fruits divided by total fruits per plot (No. Damaged fruits x 100 / Total fruits).

Three assessments were carried out at 8 DA-A, 8 DA-B and 7 DA-C for number of *Bactrocera oleae* captures in traps. 1 trap was placed in the south face of the tree, at about 1.5 m high, following the habitual practice of placement of traps for capture of *Bactrocera oleae*. DACUNEX (food attractant) and ECONEX DDVP (insecticide) were used in monitoring traps.

Trees were also checked for possible symptoms of phytotoxicity at 8 DA-A, 8 DA-B and 7 DA-C. Abbott efficacy was calculated.

The products applied were:

Trt No.	Type	Treatment Name	Form Conc	Form Unit	Form Type	Description	Rate	Rate Unit	Other Rate	Other Rate Unit	Appl Code
1	CHK	Untreated									
2	INSE	Spintor Cebo	0,024	%	CB	Spinosad	1,5	L/ha	0,36	g A/ha	ABC
3	INSE	Karate Zeon	10	%	CS	Lambda cihalotrim	125	mL/100 L	12,5	g A/100 L	ABC
	INSE	Biocebo	30	%	SL	Hydrolyzed protein	1,5	% W/V	450	g A/100 L	ABC
4	INSE	Karate Zeon	10	%	CS	Lambda cihalotrim	125	mL/100 L	12,5	g A/100 L	ABC
	INSE	Flyral	36	%	SL	Hydrolyzed protein	1,25	% W/V	450	g A/100 L	ABC
5	INSE	Karate Zeon	10	%	CS	Lambda cihalotrim	125	mL/100 L	12,5	g A/100 L	ABC
	INSE	Nulure	30	%	SL	Hydrolyzed protein	1,5	% W/V	450	g A/100 L	ABC

Results summarized as follows:

- Percentage damaged fruits: Statistically significant differences were observed between untreated and treated plots, but not between treated plots. The percentage of fruits damaged on untreated plot reached 2.5% at 8 DA-B, increased to 6.3% at 14 DA-C. Karate Zeon was sprayed in mixture with BIOCEBO or FLYRAL, applied in patch, and achieved excellent efficacies, similar to Karate Zeon when sprayed in mixture with NULURE, applied in patch, and to SPINTOR CEBO.
- Number of fallen fruits on soil due to *Bactrocera oleae*: Statistically significant differences were observed between untreated and treated plots, but not between treated plots. The number of fallen fruits on soil on untreated plot reached 30.0 at 14 DA-C. Karate Zeon was sprayed in mixture with BIOCEBO or FLYRAL, applied in patch, and achieved excellent efficacies, similar to Karate Zeon when sprayed in mixture with NULURE, applied in patch, and to SPINTOR CEBO.
- Number of *Bactrocera oleae* captures: Statistically significant differences were observed between untreated and treated plots, but not between treated plots. The number of captures on untreated plot reached 8.0 at 8 DA-A, increased to 12 at 8 DA-B and 10 at 7 DA-C. Karate Zeon when sprayed in mixture with BIOCEBO or FLYRAL, applied in patch, achieved excellent efficacies, similar to SPINTOR CEBO, and slightly higher than Karate Zeon when sprayed in mixture with LUNURE applied in patch at 7 DA-C.
- Karate Zeon was sprayed in mixture with an hydrolyzed protein applied in patch significantly decreased the damage on fruits and number of *Bactrocera oleae* compared to untreated control.
- No problems were detected when handling the experimental products.
- No symptoms of phytotoxicity were detected.

Efficacy and selectivity and evaluation of Flyral (Hydrolyzed protein 36%) and Biocebo (Hydrolyzed protein 30%) for olive fruit fly (*Bactrocera oleae* or *Dacus oleae* Dacuol) control on olive in Campania region (Italy).

Report number: PC12 BBR 34

The trial was carried out on Olive [*Olea europaea* L.var. Oliva Caiazzana], on field-grown, in Southern Italy (Campania), locality San Prisco (CE), according to an experimental design with randomized blocks, side-by-side, length-wise layout and 4 replications.

One untreated plot is served as control, Karate Zeon 1.5 is not authorized on Olive; Flyral and Biocebo are not authorized on Olive, even if their active substance was already authorized on Olive.

Nu Bait, based on Hydrolyzed protein, is authorized on Olive.

No artificial infestation was done and natural infestation of Olive fruit fly (*Bactrocera oleae* or *Dacus oleae*) was naturally present.

In the trial period infestation of not target harmful insect was observed: Black scale or Mediterranean black scale or Olive scale or Brown Olive scale or Olive soft scale (*Saissetia oleae*).

Three applications were carried out at the phenological growth stage:

- Beginning of fruit colouring (Fig 1: 81). - Fruit deep green colour becomes light green, yellowish. (BBCH 79 - 80. Application: 21/09/2012)
- Beginning of fruit colouring (Fig 1: 81). (BBCH 81. Application: 01/10/2012)
- Increasing of specific fruit colouring (BBCH 85. Application: 11/10/2012)

according to the special authorization and the study plan requirements. The applications on thesis 3,4 and 5 were made on one side of each row. Traps were placed on 30/07/2012

Summarized conclusions:

- Karate Zeon 1.5 at rate 0.125 L/ha and Biocebo at rate 1.5 L/ha ensured satisfactory protection of Olive in presence of very favourable conditions for natural infestation.
- Karate Zeon 1.5 at rate 0.125 L/ha and Flyral at rate 1.25 L/ha ensured satisfactory protection of Olive in presence of very favourable conditions for natural infestation.
- All the products have effectively contained the infestation.
- The test substances were dispersed in water without any problem and solubility was perfect. The water used as carrier was tap water taken from the farm. No problem was observed during handling of the test materials. The weather conditions in field were favourable for the development of the infestation.

CP 6.4 Summary data on trials site and application details per use (EPPO code)

Test report (1)	Trial location(2); Crop cultivar ; F/G (3); N/A (4)	Testing Unit (5)	Test method (6); Plot size; Sample Size (7)	Treatment			
				Growth stage (8)	Interval	Total number	Spray volume (L/ha)
2012023	Les Franqueses, 59 baixos E-25600 Balaguer, Lleida (ES)	Devreg Consulta SLU. EOR n° 73/11	EPPO: PP 1/135(3), PP 1/181(3), PP 1/152(3), PP 1/106(2) 1040 m ² per plot	BBCH 76 and BBCH 81. Adult	7 days intervals	2 treatments	125 L / ha
11_bio.i01	C/ El Reguero s/n 30559 Abarán - Murcia (ES)	Métodos y Servicios Agrícolas S.L. EOR n° 51/03	EPPO: PP1/152(3),PP 1/135(3), PP1/181(3), PP1/106(2) 800 m ² per plot	BBCH 81 - 83 Adult	6 days interval	3 treatments	80 L/ha
PC12 BBR 33	Caserta ViaMazzini Vico VI, n.1 - 81047 – Macerata Campania– (IT)	Biofarm s.r.l. GEP prot 2525 + 2527 (IT)	EPPO: PP1/106(2), PP1/135(3), PP1/152(3), PP1/181(3) 625 m ² per plot	BBCH 79-81 BBCH 81-85 BBCH 85-87 Adult	9 days intervals	3 treatments	100 L / ha
2012024 T1	Les Franqueses, 59 baixos E-25600 Balaguer, Lleida (ES)	Devreg Consulta SLU. EOR n° 73/11	EPPO: PP 1/108 (2), PP 1/135(3), PP 1/181(3), PP 1/152(3) 5000 m ² per plot aprox	BBCH 79 and BBCH 85. Adult	20 days interval	2 applications	125 L / ha
11_bio.i02	C/ El Reguero s/n 30559 Abarán - Murcia (ES)	Métodos y Servicios Agrícolas S.L. EOR n° 51/03	EPPO: PP 1/135(2),PP 1/181(3), PP 1/152(3), PP 1/108(2) 2000 m ² per plot aprox	BBCH 81 BBCH 85 BBCH 87 Adult	7 days intervals	3 treatments	80 L/ha
PC12 BBR 34	Caserta ViaMazzini Vico VI, n.1 - 81047 – Macerata Campania– (IT)	Biofarm s.r.l. GEP prot 2525 + 2527 (IT)	EPPO: PP1/280(1), PP1/135(3), PP1/152(3), PP1/181(3) At least 640.0 m2	BBCH 79 - 80 BBCH 81 BBCH 85 Adult	9 days intervals	3 treatments	100 L / ha

CP 6.5 Summary of data on trials site and application details per use (EPPO code)

Test report (1)	Harmful organism/ weed species or intended use	Assessed part and variable (2)	Untreated (3)	Efficacy treatments (4)				Remarks (5) All data based on damaged fruits
				Product	Standard (s)			
				BIOCEBO + insecticide rate	Conventional insecticide rate	Attractant A + insecticide rate	Attractant B + insecticide rate	
2012023	<i>Ceratitis capitata</i>	Assessed: Fruits Variable: % damaged fruits	12.50 %	1.25 %	2.75 %	0.75 %	1.75 %	
11_bio.i01	<i>Ceratitis capitata</i>	Assessed: Fruits Variable: % damaged fruits	31.78 %	0.37 %	0.25 %	0.47 %	0.47 %	
PC12 BBR 33	<i>Ceratitis capitata</i>	Assessed: Fruits Variable: % damaged fruits	57.60 %	13.5 %	9.75 %	13.3 %	14.6 %	Slightly lower efficiency but a noticeable reduction in the use of insecticide.
2012024 T1	<i>Bactrocera oleae</i>	Assessed: Fruits Variable: % damaged fruits	0.91 %	0.20 %	0.18 %	0.23 %	0.47 %	
11_bio.i02	<i>Bactrocera oleae</i>	Assessed: Fruits Variable: % damaged fruits	6.25 %	0.00 %	0.00 %	0.00 %	0.25 %	
PC12 BBR 34	<i>Bactrocera oleae</i>	Assessed: Fruits Variable: % damaged fruits	94.6 %	32.4 %	25.8 %	32.6 %	34.8 %	Slightly lower efficiency but a noticeable reduction in the use of insecticide.