STUDY TITLE

Field survey of non-target arthropods associated with *Bacillus thuringiensis* Cry1F maize in the Spanish maize agrosystem

DATA REQUIREMENTS

none

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November 12, 2002

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STATEMENT OF COMPLIANCE

This study was conducted according to permit B/ES/02/11

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Submitter: Date

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QUALITY ASSURANCE STATEMENT

Title: Field survey of non-target arthropods associated with *Bacillus thuringiensis* Cry1F maize in the Spanish maize agrosystem

Study Initiation Date: August 15, 2002 Study Completion Date: November 12, 2002

NON-GLP Study

SIGNATURE PAGE

Title: Field survey of non-target arthropods associated with *Bacillus thuringiensis* Cry1F maize in the Spanish maize agrosystem

Testing Facility: two separate field locations

Field Sites: **Montañana** (Yield trial site code SPY006). (provincia: 50; municipio: 900; poligono: 15; parcela: 00131)

Calatorao (Yield trial site code SPY007). (provincia: 50; municipio: 068; poligono: 4; parcela: 0076)

Contributors: **see title page, performing laboratories**

Study Initiation Date: August 15, 2002

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29 November 2002
Firoz Amijee, Ph.D., Study Sponsor — Date
Pioneer Hi-Bred Int'l. Inc.

29th November 2002
Angel M. Fuentes, Ph.D., Principal Investigator — Date
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TABLE OF CONTENTS

	PAGE
Statement of Compliance	2
Quality Assurance Statement	3
Signature Page	4
Table of Contents	5
Abstract	6
Scientific Terms and Abbreviations	7
Introduction	8
Materials	8
Methods	8
Results and Discussion	10
Report Conclusions	13
Archiving	13
References	14
Appendix 1.	15

Field survey of non-target arthropods associated with *Bacillus thuringiensis* Cry1F maize in the Spanish maize agrosystem

ABSTRACT

The Cry1F maize line 1507 has been modified with the *cry1*F gene from *Bacillus thuringiensis* var. *aizawai* (*B.t.*) that encodes the Cry1F protein. The Cry1F protein possesses insecticidal activity against lepidopteran pests including European corn borer (*Ostrinia nubilalis* Hubner). This line also contains a synthetic *pat* gene, derived from *Streptomyces viridochromogenes* which encodes phosphinothricin acetyl transferase (PAT) that confers tolerance to the herbicide glufosinate-ammonium.

This observational study was conducted in two locations where permits were issued to grow maize hybrids containing event TC1507. The experiments planted at both locations were small-plot experiments intended for agronomic purposes, and we took the opportunity to observe potential effects on the Spanish beneficial insect complex. Therefore, the experimental design used for these plantings precludes any statistical comparisons of arthropod density between conventional and transgenic (1507) hybrid entries. However, these plantings did provide an opportunity to collect information on the abundance of several beneficial arthropods in areas where future field studies addressing the Spanish maize agrosystem may take place.

SCIENTIFIC TERMS AND ABBREVIATIONS

Maize Developmental Stages:

V9	9^{th} leaf
R1	silking or pollen shed
R2	blister stage grain
R4	dough stage grain

INTRODUCTION

A. Background

The Cry1F maize line 1507 has been modified with the *cry1*F gene from *Bacillus thuringiensis* var. *aizawai* (*B.t.*) that encodes the Cry1F protein. The Cry1F protein possesses insecticidal activity against lepidopteran pests including European corn borer *Ostrinia nubilalis* Hubner. Maize line 1507 also contains a synthetic *pat* gene, derived from *Streptomyces viridochromogenes*, which encodes the PAT protein (enzyme) that confers tolerance to the herbicide glufosinate-ammonium. The non-GM control maize line has the background genetics representative of the test substance line but is not genetically modified and therefore, does not express the Cry1F or PAT proteins.

B. Purpose

The purpose of this study was to measure the abundance of beneficial arthropods at two field test locations where plots of *Bacillus thuringiensis* var. *aizawai* (*Bt*) Cry1F maize and a non-GM isoline maize were grown. These data highlight the relative abundance of important families of predatory insects and spiders (pooled Arachnida) over time and will help increase the sampling efficiency for subsequent research on the potential impacts of 1507 maize on the beneficial arthropod community in a typical Spanish maize agrosystem.

MATERIALS

A. Test substance

Maize research plots containing hybrid maize including six entries from conventional non-GM maize and four entries from 1507 maize hybrids with similar genetic backgrounds as four of the conventional entries.

B. Test system

These observations were recorded from within two similar experiments planted at different locations; see page 4 Field Sites.

METHODS

A. Summary of experimental design

This was an observational study overlaid on an existing experiment.

Ten entries were tested in each location, and each site had its own unique entry randomization. Six entries were conventional non-GM maize and four entries were 1507 maize hybrids with similar genetic backgrounds as four of the conventional entries. Entries were randomized within blocks, and two replicate blocks were planted adjacent to each other. Each entry was planted as a four-row plot, and plots were separated by an alley distance of approximately 1 meter. The entire trial was surrounded by a minimum of four rows of conventional maize hybrid of similar relative maturity. The surface area of the trial, including the four surrounding rows, was approximately 861m^2.

All entries were planted at a seeding rate of approximately 50 seeds per row. Each row was approximately 7.5 meters in length. Seed spacing within each row was approximately 15 cm. Spacing between rows was approximately 75 cm.

B. Field Trial

1. Agronomic practices

Agronomic practices consistent with maize production for the area were used to produce the crop, and all applications of fertilizer, water and herbicides were made to the entire maize planting.

CULTURAL PRACTICE CALENDAR IN: SPY007 (Calatorao)

Date	Intervention
5/30/2002	Fertilizer application (15-15-15) Dosis: 300 Kg/Ha
6/3/2002	Flooding Irrigation
6/7/2002	Field preparation (Cultivation)
6/12/2002	Drill
7/3/2002	Herbicide application (Elite M - Nicosulfuron 4%)
7/9/2002	Flooding Irrigation
7/26/2002	Hand weeding
7/29/2002	Flooding Irrigation
8/5/2002	Fertilizer application (N - 33,5%) Dosis: 320 Kg/Ha
8/6/2002	Hand weeding
8/6/2002	Herbicide application (Elite M - Nicosulfuron 4%)
8/12/2002	Flooding Irrigation
9/17/2002	Flooding Irrigation
9/5/2002	Hand weeding

CULTURAL PRACTICE CALENDAR IN: SPY006 (Montañana)

Date	Intervention
6/3/2002	Field preparation (Cultivation)
6/7/2002	Fertilizer application (15-15-15) Dosis: 300 Kg/Ha
6/10/2002	Flooding Irrigation
6/13/2002	Drill
7/5/2002	Flooding Irrigation
7/8/2002	Herbicide application (Elite M - Nicosulfuron 4%)
7/26/2002	Hand weeding
7/28/2002	Flooding Irrigation
8/1/2002	Fertilizer application (N - 33,5%) Dosis: 320 Kg/Ha
8/6/2002	Herbicide application (Elite M - Nicosulfuron 4%)
8/6/2002	Hand weeding
8/14/2002	Flooding Irrigation
8/24/2002	Flooding Irrigation
9/5/2002	Hand weeding
9/9/2002	Flooding Irrigation

2. Climate

Weather conditions were not documented for this experiment.

3. Sampling

Observations were collected using visual inspection of 20 plants per plot. Arthropods were counted if they were observed interacting with the plant. Only plants from the inner two rows of each plot were sampled, and plants near the end of rows were not sampled. Samples were collected at a uniform time of day among dates and locations. Samples were taken according to the date and maize developmental schedule below:

Maize Developmental Stage	Calatorao	Montañana
V9	15 August	16 August
R1	28 August	27 August
R2	5 September	4 September
R4	23 September	22 September

C. Control of bias

Sample points within plots were randomly selected.

D. Statistical analysis

This observational study reports the average number of important beneficial arthropods observed per 20 plants sampled using visual observations. As mentioned earlier, the experiment design for these agronomic trials precludes statistical comparisons, however the observations do provide useful comparisons of arthropod density between conventional and 1507 maize hybrids.

RESULTS AND DISCUSSION

Arthropod abundance

Observations were made on six insect families with members that belong to the functional group of predator insects. These families included Coccinellidae, Nabidae, Anthocoridae, Chrysopidae, Pentatomidae and Lygaeidae, and all of these are important predator families in maize systems throughout the world.

Trends for all family-specific and stage abundance observations between locations had correlation coefficients greater than 0.98 for V9, R1, R2 and R4 sample dates. Observations from either location were representative of both locations for conditions at these two locations and during this sampling interval.

There was a general trend for larger arthropod densities near the R1 and R2 sample dates. The abundance of fresh pollen and silk during this period likely explains the change in arthropod abundance.

Anthocoridae were the most abundant predator observed across dates at both locations. Nymphs and adults were most abundant during the R1 sample period, and relatively high numbers of adults were present at all sample dates (Fig. 1). Members of this family recently have gained attention regarding their utility in the risk assessment process for plant incorporated protectants similar to 1507 maize (US EPA 2002).

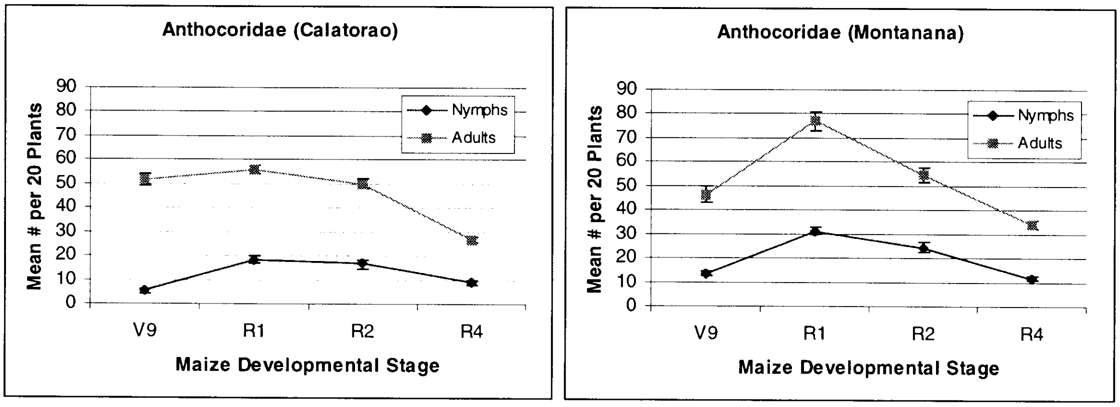

Figure 1. Mean and standard errors for Family Anthocoridae at both locations.

Spiders were the second most abundant predatory arthropod sampled at both locations, and their density declined after the R1 maize developmental stage (Fig. 2).

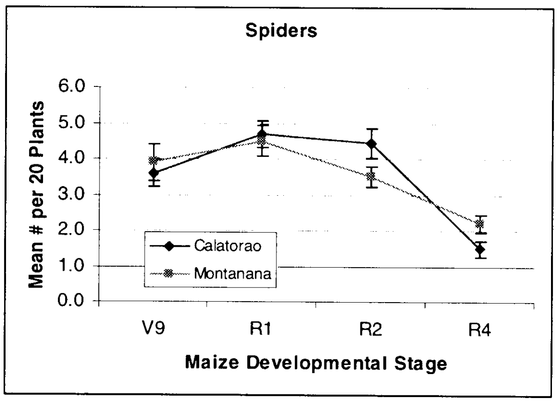

Figure 2. Mean and standard errors for pooled observations on spiders.

Members of the family Nabidae were the third most abundant predatory arthropod. Adult and nymphs were observed in similar numbers and there was a general trend for gradually increasing density over the four sample periods (Fig 3.).

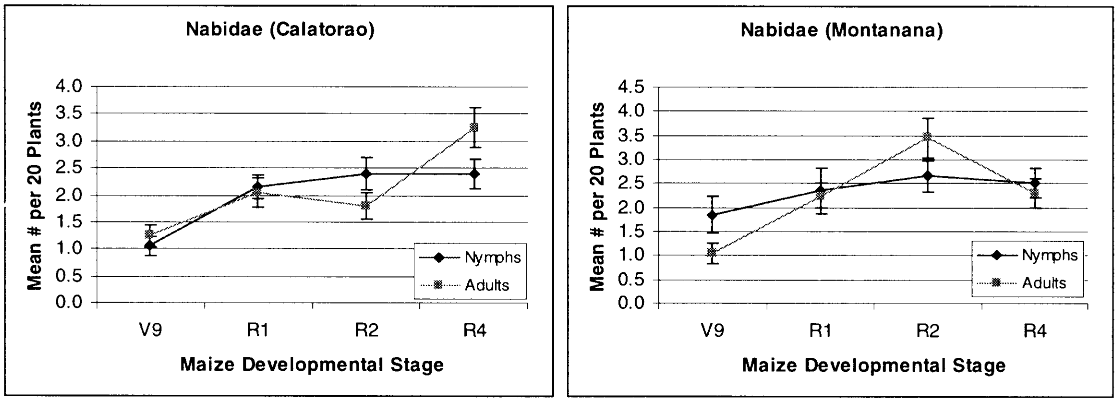

Figure 3. Mean and standard errors for Family Nabidae at both locations.

Members of the Chrysopidae were less abundant overall. Adults and larvae were present, but infrequent at the earliest sample point and numbers declined thereafter (Fig. 4). However, Chrysopid eggs were observed throughout the entire sample period at higher densities than adults and larvae.

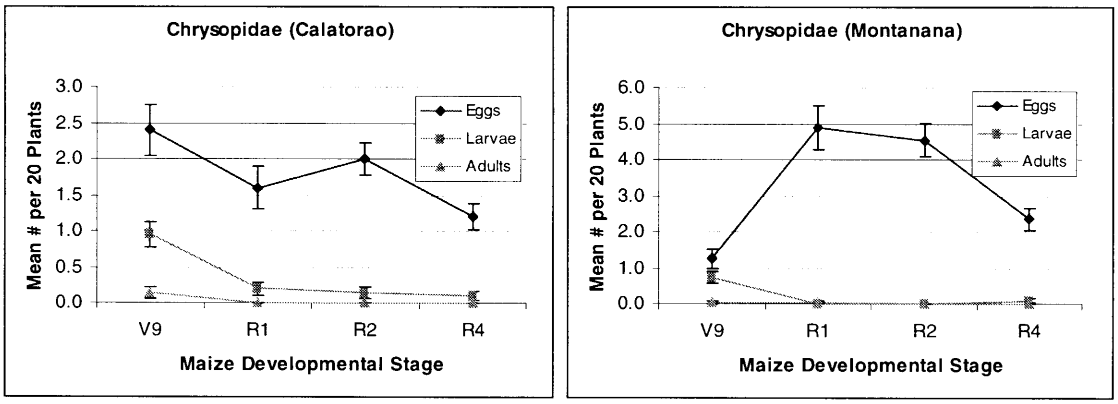

Figure 4. Mean and standard errors for Family Chrysopidae at both locations.

Members of the Family Coccinellidae were present throughout the sampling period at both locations (Fig 5.). Estimates were relatively low and these observations did not show an obvious or consistent trend.

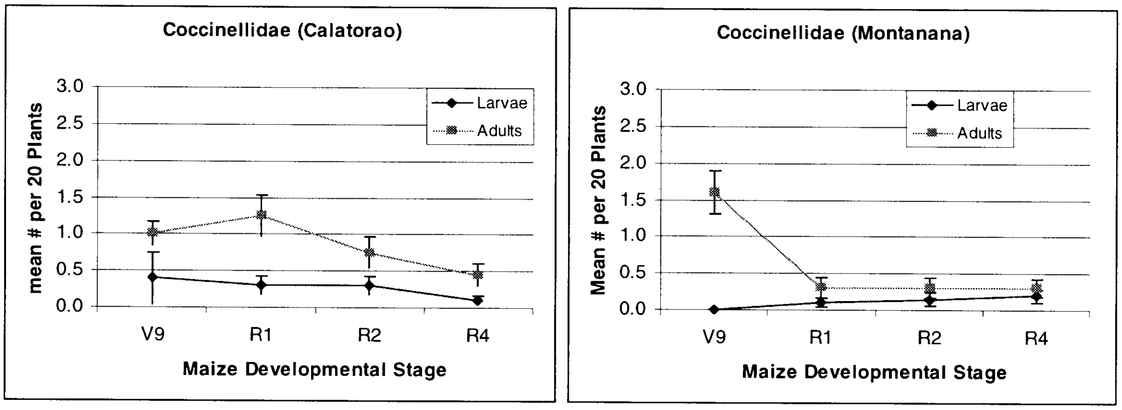

Figure 5. Mean and standard errors for Family Coccinellidae at both locations.

Members of the families Lygaeidae and Pentatomidae were the least abundant of the sampled groups (Fig. 6). The abundance of both families increased during R1 and it is not clear if visual observations are an inefficient sampling technique or if densities were very low.

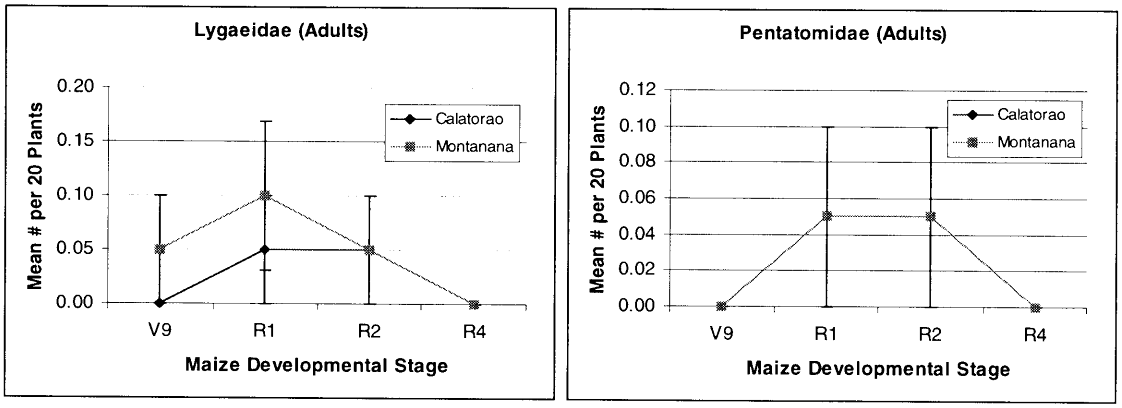

Figure 6. Means and standard errors for the families Lygaeidae and Pentatomidae at both locations.

Between location estimates follow similar trends, and this increases confidence in the relative importance of each beneficial arthropod group in the Spanish maize agrosystem at these locations. These observations are from a single growing season, and the relative rank of each group likely will change with variation in environmental conditions.

Between Maize-Type Observations

Hybrid entries were pooled by type: conventional control or 1507 maize. Means for control hybrids result from pooling estimates from two official check hybrids and four hybrids that are isogenic to the 1507 maize hybrids. Means for 1507 maize result from pooling estimates from four 1507 maize hybrids expressing the Cry1F protein. Means and standard errors for both locations are listed in Appendix 1. Table 1.

Abundance of these beneficial arthropod groups followed similar trends in both maize types. Moreover, the trends across four sample periods for both maize types each are highly correlated (>

0.99) with the trend of pooled (control and 1507 maize, Figs. 1-6) estimates, and this result is consistent across both locations. These results do not highlight any potential differences in how members of these beneficial arthropod groups use conventional and 1507 maize. These are not conclusive results on the potential effects of 1507 maize on the Spanish beneficial insect complex. However, they do support previous risk assessment studies of non-target organisms in 1507 maize where favorable conclusions have been reached (Higgins 1999, Vernier *et al.* 2000).

REPORT CONCLUSIONS

Members of the sampled beneficial arthropod groups were most abundant at or soon after pollen shed. Sampling programs for future studies should consider focusing on this period of crop development because of high arthropod abundance and the potential for increased precision of field-based estimates. Members of Anthocoridae, Spiders, and Nabidae ranked first, second and third in overall abundance across sample dates and locations. Again, high numbers facilitate sampling precision in field studies and the relative importance of these three groups in a risk assessment were considered. Members of the other groups may have higher relative importance in the maize agrosystem, and this may have been masked by a single season of observations. The visual observations overlaid on this field experiment did not highlight any potential differences in how members of these beneficial arthropod groups use conventional and 1507 maize.

ARCHIVING

Raw data and the original copy of the final report will be filed in the Pioneer Hi-Bred Int'l. Inc. Regulatory Science and Registration Department archives, Johnston, Iowa, USA.

REFERENCES

US Environmental Protection Agency. 2002. Scientific Advisory Panel Meeting Minutes 2002-05 (http://www.epa.gov/scipoly/sap/2002/august/august2002final.pdf)

Higgins, L. S. 1999. Field Survey of Beneficial Arthropods Associated with *Bacillus thuringiensis* Cry1F Maize. MRID#45020113

Vernier A.V., V. Berrone and C. Ulvé. 2000. Field Study of Non-Target Arthropods associated with *Bacillus thuringiensis var. aizawai* Cry1F maize. Notification to market products containing genetically modified organisms in accordance with Directives 90/220/EC and 2001/18/EC: 1507 maize (Notification Ref. C/ES/01/01)

APPENDIX 1.

Maize Stage	Maize Type*	Spider	Coccinellidae		Nabidae		Anthocoridae		Chrysopidae			Pentatomidae		Lygaeidae	
			Larvae	Adults	Nymphs	Adults	Nymphs	Adults	Eggs	Larvae	Adults	Nymphs	Adults	Nymphs	Adults
								Calatorao, Spain							
V9	control	3.6 ± 0.47	0.7 ± 0.58	0.8 ± 0.21	1.1 ± 0.29	1.3 ± 0.26	5.3 ± 1.06	49.9 ± 3.04	2.5 ± 0.48	0.8 ± 0.13	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
V9	1507	2.9 ± 0.60	0.1 ± 0.03	1.1 ± .020	1.1 ± 0.17	1.3 ± 0.28	4.8 ± 1.05	42.7 ± 6.63	1.9 ± 0.43	1.0 ± 0.29	0.2 ± 0.12	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R1	control	4.5 ± 0.56	0.4 ± 0.19	1.3 ± 0.33	2.3 ± 0.22	2.3 ± 0.37	19.2 ± 2.01	55.9 ± 2.43	1.6 ± 0.43	0.3 ± 0.14	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00
R1	1507	4.2 ± 0.55	0.1 ± 0.09	1.2 ± 0.37	1.9 ± 0.34	1.5 ± 0.31	14.4 ± 2.62	46.9 ± 5.75	1.5 ± 0.28	0.2 ± 0.09	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R2	control	4.3 ± 0.64	0.3 ± 0.19	0.7 ± 0.22	2.8 ± 0.37	1.7 ± 0.36	15.4 ± 1.18	51.3 ± 2.98	2.0 ± 0.30	0.2 ± 0.11	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00
R2	1507	4.0 ± 0.48	0.2 ± 0.11	0.9 ± 0.31	1.7 ± 0.33	1.7 ± 0.24	16.4 ± 2.10	41.4 ± 4.62	1.7 ± 0.31	0.1 ± 0.09	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R4	control	1.6 ± 0.31	0.1 ± 0.08	0.2 ± 0.11	2.5 ± 0.31	3.3 ± 0.46	9.7 ± 1.04	27.8 ± 1.53	1.2 ± 0.21	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R4	1507	1.6 ± 0.33	0.2 ± 0.09	0.8 ± 0.21	1.9 ± 0.40	2.6 ± 0.53	8.0 ± 1.12	24.1 ± 2.90	1.2 ± 0.27	0.1 ± 0.09	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
								Montañana, Spain							
V9	control	3.9 ± 0.54	0.0 ± 0.00	2.1 ± 0.38	2.0 ± 0.58	1.3 ± 0.28	13.4 ± 1.36	45.8 ± 4.47	1.6 ± 0.31	0.8 ± 0.22	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
V9	1507	3.1 ± 0.81	0.1 ± 0.04	0.7 ± 0.22	1.6 ± 0.29	0.9 ± 0.25	11.7 ± 1.44	38.6 ± 6.18	0.9 ± 0.29	0.6 ± 0.20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R1	control	4.6 ± 0.60	0.2 ± 0.11	0.4 ± 0.23	2.8 ± 0.72	2.2 ± 0.34	31.8 ± 2.11	78.5 ± 5.82	5.1 ± 0.88	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.2 ± 0.11
R1	1507	3.7 ± 0.55	0.0 ± 0.00	0.3 ± 0.12	1.3 ± 0.32	2.0 ± 0.36	23.0 ± 4.25	60.9 ± 7.73	3.7 ± 0.74	0.2 ± 0.11	0.1 ± 0.04	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R2	control	4.0 ± 0.37	0.1 ± 0.08	0.3 ± 0.18	2.5 ± 0.42	3.2 ± 0.63	24.0 ± 2.69	53.3 ± 3.49	4.5 ± 0.63	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00
R2	1507	2.6 ± 0.34	0.2 ± 0.12	0.3 ± 0.18	2.3 ± 0.46	3.1 ± 0.46	22.1 ± 3.28	50.2 ± 6.20	4.0 ± 0.62	0.1 ± 0.04	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R4	control	2.2 ± 0.34	0.2 ± 0.11	0.4 ± 0.19	2.3 ± 0.39	2.3 ± 0.48	9.7 ± 0.97	36.2 ± 2.86	2.4 ± 0.43	0.2 ± 0.11	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R4	1507	2.0 ± 0.31	0.2 ± 0.11	0.2 ± 0.10	2.5 ± 0.40	2.2 ± 0.32	14.0 ± 1.52	29.6 ± 3.31	2.3 ± 0.38	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

* control = non-transgenic hybrid entries (2 official check hybrids and 4 hybrids that are isogenic to the TC1507 hybrids); 1507 = transgenic hybrid entries (4 hybrids expressing the Cry1F protein).

Table 1. Means and standard errors of control and 1507 hybrids for Calatorao and Montañana. Control estimates result from pooling 20 observations per plot, 2 plots per hybrid entry and 6 entries per location (240 individual plant observations per location), and 1507 estimates result from pooling 20 observations per plot, 2 plots per hybrid entry and 4 entries per location (160 individual plant observations per location).