ENVIRONMENTALLY SAFE STRATEGIES TO CONTROL THE EUROPEAN GRAPEVINE MOTH, *LOBESIA BOTRANA* (DEN. & SCHIFF.)**, IN THE DOURO DEMARCATED REGION.**

STRATEGIES RESPECTUEUSE DE L'ENVIRONNEMENT POUR CONTROLER L'EUDEMIS DE LA VIGNE, *LOBESIA BOTRANA* (DEN. & SCHIFF.), DANS LA REGION DU DOURO

Cristina CARLOS[1,2*], Fátima GONÇALVES[2], Susana SOUSA[1], Juliana SALVAÇÃO[2], Lav SHARMA[2], Rui SOARES[3], José MANSO[4], Márcio NÓBREGA[4], Álvaro LOPES[3], Sérgio SOARES[3], José ARANHA[2], Claire VILLEMANT[5], Guilhermina MARQUES[2] and Laura TORRES[2]

[1]ADVID – Association for the Development of Viticulture in the Douro Region, Quinta de Santa Maria, Apt. 137, 5050-106 Godim, Portugal
cristina.carlos@advid.pt
[2]CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, 5001-801, Vila Real, Portugal
[3]Real Companhia Velha- R. Azevedo Magalhães, n.º 314 Oliveira do Douro, 4430-022 Vila Nova de Gaia
[4]Sogevinus Quintas S.A.- Avenida Diogo Leite 344, Santa Marinha, 4400-111 Vila Nova de Gaia
[5]Muséum National d'Histoire Naturelle, UMR 5202 CNRS-MNHN. CP 50 Entomologie. 45 rue Buffon, 75005 Paris

ABSTRACT

In Douro Demarcated Region (DDR), the European Grapevine Moth (EGVM) is a key pest of commercial vineyards. The development of effective and environmentally safe control strategies against this pest in DDR, is a major objective of EcoVitis project, that join Real Companhia Velha, Sogevinus Quintas S.A., Association for the Development of Viticulture in the Douro Region and University of Trás-os-Montes and Alto Douro. This objective is being examined by two main approaches: pheromone-based mating disruption (MD) and enhancement of conservation biological control of the pest. The MD technique has been applied in DDR to control the EGVM, since 2000, mainly using ISONET-L dispensers (Shin-Etsu Chemical Co.), increasing from an initial 9 ha to 214 ha in 2012. This technique has proved to reduce the levels of the pest, however, in some cases it did not successfully reduce infestation below the tolerance threshold, mainly in the third generation, and insecticides had to be applied. Some constrains have been identified, such as the high biotic potential of the pest, the high summer temperatures, the orography (high steepness) and the mix of vineyards and untreated habitats, where alternative hosts (e.g. *Daphne gnidium* L.) are common. In order to avoid some of these constrains, one of the goals of EcoVitis is to test a new dispenser loaded with higher dosis of pheromone. Studies on EGVM biological control agents are being carried at DDR since 2002. To date, seven species of Hymenopteran parasitoids have been identified being *Elachertus affinis* (Masi), *Brachymeria* sp., *Campoplex capitator* (Aubert) and *Dibrachys cavus* (Förster) the most commons; also several species of entomopathogenic fungi, mainly from the genera *Beauveria* and *Metarhizium* are being isolated. Under EcoVitis it is hypothesized that we can enhance biological control by arthropod enemies and by native entomopathogenic fungi by manipulating the ecological infrastructure of local farms.

Key Words: Functional biodiversity, landscape management, mating disruption, conservation biological control

1- INTRODUCTION

The 'Douro Demarcated Region' (DDR) is considered the oldest controlled winemaking region in the world. It covers an area of approximately 250,000 ha, of which some 45,000 ha are planted with vines, and the most representative and well-preserved part of this region (about 10% of the total area), named as the 'Alto Douro Wine Region' or just the 'Alto Douro, is a UNESCO World Heritage Site for its unique character as an 'evolved continuing cultural landscape'. Unlike other wine growing areas in the world, the Alto Douro landscape offers a strong potential from the standpoint of biodiversity, due to the occurrence of a significant area of non-crop habitats (e.g. woodland remnants, grassy slopes, or terraces with natural vegetation and dry stone walls). A quarter of the scrub consists of terraces that were abandoned after the devastation of phylloxera in the late 19th century. These "mortuaries" of former vineyards are now overrun with wild flora, whose importance for the conservation of a wide range of biota in agricultural landscapes is known. However, agricultural intensification as practiced over centuries in the region, has led to habitat destruction as well as fragmentation of remaining habitat, with expected negative effects on the occurrence of species and thus on biodiversity. The European Grapevine Moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is a key-pest of vineyards in Alto Douro, infesting up to 50% of grapes at harvest. The objective of EcoVitis project, is to examine innovative plant protection techniques to reduce pest damage with minimal environmental impact, by two main approaches: an enhanced role of natural enemies (Arthropoda, Fungi and Nematoda), and pheromone-based mating disruption. Our interest for understanding the landscape effects on *L. botrana* natural antagonists arise from prospects of giving first inputs for landscape-based conservation biological control of this key pest of vineyards. We aim to improve results using each technique, as well as integrate them into one workable strategy.

2- MATERIAL AND METHODS

The study was carried in five pilot farms, i.e. Quinta de S. Luíz (Sogevinus Vinhos SA), Quinta das Carvalhas, Quinta dos Aciprestes, Quinta da Granja e Quinta do Cidrô (Real Companhia Velha). Except the last two farms all are located in the *Alto Douro Vinhateiro* classified Area. The landscape is dominated by a matrix of Mediterranean woodland, scrubland with a varying degree of fragmentation caused by the interspersion of agricultural land uses (mainly vineyards and olive groves) and urban areas.

2.1 - Landscape analysis

The landscape of the five farms was categorised in habitats according to its land use and digitised by means of a geographical information system (ArcGIS 9.3). Shannon-Wiener biodiversity index (H') and evenness index (E) were calculated using the Patch Analyst for ArcGIS.

2.2 – Enhancement of conservation biological control of the pest

2.2.1 - Parasitism

To check the presence of parasitoids, larvae and pupae of *L. botrana* were sampled from several plots of five farms, namely Carvalhas, Aciprestes, Granja, Cidrô and S. Luíz, from May (first generation) to October (third generation) and incubated in the laboratory (22ºC; RH: 65±10%, photoperiod 16:8 (L:D)) for quantification of parasitism and identification of parasitoids emerged to specie, when possible. Species abundance (N), richness (S), Shannon-Wiener biodiversity index (H') and evenness index (E) were calculated to provide information on parasitoid diversity in each location.

The relationship between these parameters (percentage of parasitism, H' and E) and the landscape indexes was calculated using Spearman's (non-parametric) correlation analysis. Calculations were done with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

2.2.2 – Entomopathogenic agents

To check the presence of entomopathogenic fungi and nematodes, soil samples are being collected from the six different farms during fall 2012 and were baited with six larvae of *Tenebrio molitor* ((Linnaeus) (Coleoptera: *Tenebrionidae*)) in a 9 cm Petri plates, inside a humid chamber at 25ºC, for up to three weeks. Petri plates were checked for presence of cadavers every 3-4 days and subsequently kept in another 9 cm Petri plate containing cotton with filter paper, both sterilized, for one week to enhance the growth of entomopathogens. Cadavers were then transferred into a selective media i.e., Dichloran Rose-Bengal Chloramphenicol Agar (Merck Chemicals, Germany) supplemented with 1 ml streptomycin sulphate (0.5 g/10 ml (w/v)) per litre. According to the symptoms, i.e., emergence of nematodes or growth of fungi, pure cultures were maintained, as described by Grewal *et al*., (2005) and Meyling (2007), respectively.

2.3 – Mating disruption

The MD technique was applied using ISONET-L dispensers (Shin-Etsu Chemical Co.) The experiments took place in Carvalhas during 2011 and 2012. In 2011 it was carried in an area of 14,1 ha of traditional vineyard with long history of *L. botrana* infestations. In 2012 the experiments were extended to more 22,3 ha totalizing about 36,4 ha. Four plots without MD treatment located in the same farm but apart from the experiment were used as control plots; whenever possible plots with similar structure, variety or varietal sensibility and comparable history of pest damage were chosen, as recommended by the European and Mediterranean Plant Protection Organization (EPPO, 2008). For adult monitoring, during mid-March a network of pheromone traps loaded with *L. botrana* pheromone was set up covering all experimental plots. Traps were placed in the center of each plot, and were examined weekly for captured males. Application density was determined based on the size of the treated area, wind direction, planting system, history of *L. botrana* infestations in the area and type of borders and was of 480 dispensers per ha in 2011 and between 480 and 530 in 2012. The application was made in order to strengthen the higher quotas. Due to high risk of pest infestation, a spray with metoxiphenozide (Runner) was done in the control plots during the second generation of 2011 in the beginning of July, as well as in 2012 in the third generation in two pheromone-treated plots and in one control plot in the last week of August.). Inflorescence or grape infestation was assessed in each plot and generation by inspecting a sample of between 50 and 100 randomly selected inflorescence or grape. This procedure was normally done after the flight peak. In the first generation the level of infestation was expressed as the number of nests per 100 inflorescences, while in the second and third generation, the infestation level was expressed as the number of infested grape clusters per 100 grape clusters. The efficacy of treatment was measured by comparing either pheromone trap catches and inflorescence and grape clusters infestation in MD control plots with the control plot that were closest to the plot. Results were expressed as the percentage of male disorientation and the reduction of infestation in the MD.

3- RESULTS AND DISCUSSION

3.1- General landscape analysis

Aciprestes and Carvalhas have the highest H'and E indexes of landscape (Table I) which means that these two farms have a landscape more diverse and better distributed. Instead, Granja have the lowest indexes.

3.2- Parasitism of *Lobesia botrana*

The percentage of parasitism was higher in the first generation, being over than 20% in 35.6 % of the samples and over than 40% in 24.4% of the samples (Table II). Concerning the analysis of the parasitism obtained in each farm, Aciprestes and Carvalhas were the two farms were it was possible to find a high number of parasitized individuals (46.4 and 33.9%, respectively) (Table III) whereas in Granja only 2.9% of larvae were parasitized.

Thirteen different morpho-species of parasitoids were collected from *L. botrana* (Table IV). Three of them were identified at the level of specie, namely *Elachertus affinis* (Masi) (Hym.: Eulophidae), *Campoplex capitator* (Aubert) (Hym.: Ichneumonidae) and *Ascogaster quadridentata* (Wesmael) (Hym.: Braconidae). One was identified to genera, *Brachymeria* sp. (Hym.: Chalcididae). The most abundant were by decreasing order: *E. affinis*, representing 80.8% of the individuals obtained, *C. capitator*, with 11.8%, *Brachymeria* sp., with 2.4%, and *A. quadridentata* with 1.8%.

In relation to the diversity of parasitoids (Shannon and Eveness indexes), the two farms with more ratio of vineyard (Granja and S. Luiz) were in fact the two where this two indexes were higher (Table IV).

The percentage of parasitism was positively correlated with the Shannon index calculated for the landscape (r_{ho} = 0.900, p = 0.037) which means that more diversified landscapes (i.e., Aciprestes and Carvalhas) have a positive impact on the parasitism of *L. botrana*. However, concerning the diversity of species found, these two landscapes did not attract more diverse parasitoids. Instead of that, the landscapes dominated by vineyards have recorded a more balanced complex of species i.e. a higher Eveness Index (r_{ho}=1), than others landscapes with more Ecological Compensation Areas (r_{ho} = -1).

No significant correlations could be found between diversity indexes of parasitoids and landscape. Other studies have also shown no correlations (Feller and Mathis, 1997) and even negative correlations (Hill *et al*., 1995) between these parameters, as a consequence of fragmentation which cause the disruption of some biological processes such as dispersal and resource acquisition (Saunders *et al*., 1991).

In conclusion, it was shown that simple landscapes have lower parasitism rates but a more diverse complex of parasitoids.

3.3- Assessment of presence entomopathogenic fungi and nematodes on soil

Different entomopathogenic fungi and nematode species were found in the studied vineyards based on preliminary findings. Some fungal isolates were identified as *Beauveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* (Metsch.) Sorokin two well-known entomopathogens, but other fungal species have been found. Molecular identification and toxicity bioassays of the species are in process.

3.4- Mating disruption

In both years, three flight periods were observed during each season, whose total number of captures is presented in table V. In general, capture in the MD plots was lower than in control plots. During the first flight, and in both years, the higher number of captures in MD plots occurred mainly between the last two weeks of March and the first of April. This apparently non-efficacy of the method could have been due to the difficult in maintaining the pheromone cloud surrounding the vines, because the absence of foliage that retain the pheromone (Karg and Sauer, 1997). Trap captures were 94.43±1.26% lower in the MD plots compared with the control in 2012, and 98.93±0.66% lower in 2011, in the first flight. Concerning to second and third flights, the reduction of captures were respectively of 97.80±1.47 and 98.99±1.01%, in 2012. In 2011, during the third flight, no reduction of captures was found in MD plots in relation to the corresponding control plot. However these results should be analyzed with caution since in this control plots an insecticide spray was done.

Nevertheless this observed reduction in captures does not resulted in a comparable reduction of damages. In fact, the capture of even a few males in pheromone traps indicates a high risk of MD unsuccessful since that, the amount of pheromone needed to disrupt male orientation to pheromone traps is lower than the amount needed to disrupt mating (Ioriatti *et al*., 2011).

Thus, in 2011 the infestation reduction was null in the first and third generations whereas in the second was very low or even null in some plots (Table VI). In 2012, the method seems to had been more effective although not even providing satisfactory results (Table VII). Three reasons could explain for the better results found in the last year: a lower population pressure which decrease the probability of occasional mating (Mochos *et al*., 2004); the increase of the treaded area, which reduced the possibility of mated females migration from surrounding

vineyards (Mochos *et al.*, 2004); and the accumulated effect of the method, when applied in successive years in the same area (Anfora, 2005; Mochos *et al.*, 2004; Carlos *et al.*, 2010).

In 2012, the levels of infestation in MD plots were in general lower than the occurred in control plots and below the threshold economic level. The insecticide spray done this year was justified by the high number of captures observed in a period in which the threshold level of 5% was being approached or surpassed in control plots and by the high number of captures found in MD plots with important historical infestations. In 2012, MD seems to have been less effective against the third generation as shown by data collected at harvest. This could have been due to the almost exhaustion of the pheromone in the dispensers (Mochos *et al.*, 2004). On the other hand, the insecticide spray done during the second generation in the control plots allowed a decrease of infestation in these plots in 2011, which on average was lower than in MD plots (6.18±1.40 in the first generation and 24.85±21.40 in the second). Some authors have shown that the combined use of insecticide and MD can be used to reduce population density of *L. botrana* to a level where MD alone can be used to control this pest (Louis et al., 2002; Akyol and Aslan, 2010). However in the present study, the insecticide spray could be better have been done in the first generation, as referred by Louis and Schirra (2001), in order to reduce the population and improve the effectiveness of MD in the following generations (Mochos *et al.*, 2004; Charmillot, 1992).

Also the wind exposure in addition with the geography and topography (slope) has an important role on the release rate of pheromone as well as in the maintenance of the cloud in the plot. The dispensers were placed on the assumption that the pheromone cloud tends to down the hill. However, in Alto Douro region the wind doesn't have a singular patter of direction, having, normally during the morning a descendent movement and during the afternoon, due to the presence of the river, an ascendant movement through by so called river breezes.

6- REFERENCES

Akyol B., Aslan M., 2010. Investigations on Efficiency of Mating Disruption Technique Against the European Grapevine Moth (*Lobesia botrana* Den. Et. Schiff.) (Lepidoptera; Tortricidae) in Vineyard, Turkey. *J. Anim. Vet. Adv.,* **9** (4), 730-735

Carlos C., Alves F., Torres L., 2010. Constraints to the application of mating disruption against *Lobesia botrana* in Douro Wine Region. *Congresso Internacional de Viticultura de Montanha, Castiglione di Sicilia, 12-14 Maio 2010.*

Charmillot P.J., 1992. Mating disruption technique to control grape and wine moths: general considerations. *Bull. OILB/SROP,* **15**(5), 113-116.

EPPO, 2008. *Mating disruption pheromones. EPPO* Bulletin, **38**, 322–325.

Ioriatti C., Anfora G., Tasin M., Cristofaro A., Witzgall P., Lucchi A., 2011. Chemical Ecology and Management of *Lobesia botrana* (Lepidoptera: Tortricidae). *J. Econ. Entomol.,* **104**(4), 1125-1137 (2011).

Karg G., Sauer A.E. 1997. Seasonal Variation of Pheromone Concentration in Mating Disruption Trials Against European Grape Vine Moth *Lobesia botrana* (Lepidoptera: Tortricidae) Measured by EAG. *J. Chem. Ecol,* **23**(2), 481-501

Louis F., Schirra K.J., 2001. Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae) in vineyards with very high population densities. *IOBC wprs Bulletin,* **24** (2), 75-79.

Louis F., Schmidt-Tiedemann A., Schirra K.J., 2002. Control of *Sparganothis pilleriana* Schiff. and *Lobesia botrana* Den. & Schiff. in German vineyards using sex pheromone-mediated mating disruption. *IOBC wprs Bulletin,* **25**, 51-58.

Moschos T., Souliotis C., Broumas T., Kapothanassi V., 2004. Control of the European Grapevine Moth *Lobesia botrana* in Greece by the Mating Disruption Technique: A Three-Year Survey. *Phytoparasitica,* **32**(1), 83-96

Grewal P.S., Ehlers R.U., Shapiro-Ilan D.I., 2005.Nematodes as Biocontrol Agents. CABI, New York, USA.

Meyling N.V., 2007. "Methods for isolation of entomopathogenic fungi from the soil environment". Laboratory Manual, Faculty of Life Science, University of Copenhagen, Denmark.

Apresentado sob a forma de apresentação oral no 18º Internacional Symposium GIESCO, Porto, 7-11 Julho 2013

Hill J.K., Hamer K.C., Lace L.A., BanhamW.M.T., 1995. Effects of selective logging on tropical forest butterflies on Buru, Indonesia. *Journal of Applied Ecology,* 32, 754–760.

Feller I.C., Mathis W.N., 1997. Primary herbivory by woodboring insects along an architectural gradient of Rhizophora mangle. *Biotropica*, 29, 440–451.

Saunders D.A., Hobbs R.J.,Margules C.R., 1991. Biological consequences of ecosystem fragmentation: a review. *Conservation Biology,* **5**, 18–32.

Table I- General description of the landscape of the six farms included in this study

Farms	Aciprestes	Granja	Cidrô	S. Luíz	Carvalhas
Total area (ha)	144,0	239,2	242,90	131,80	311,9
Area with vineyards (%)	53,5	75,2	55,7	69,3	39,2
Area with potential ECA * (%)	37,0	16,8	35,5	19,5	50,5
Diversity Index (Shannon)	**1,29**	0,90	0.98	1,09	**1,57**
Equitability Index (Eveness)	**0,66**	0,46	0,55	0,53	**0,75**

As Potential ECAs (Ecological Compensation Areas) we include forest, woodland, shrubland, water, cultivated or abandoned orchards (including olive groves), gardens, and embankments with natural vegetation. We do not include concrete structures, roads or urban areas.

Table II- Percentage of parasitism observed in each of the three generations of *Lobesia botrana*

Gen.	Nb samples	Nb larva/pupa collected	Nb larva/pupa parasitized	Parasitism (%)	% samples > 20% Parasitism	% samples > 40% Parasitism	Range (%)
1rst	45	1475	319	21.6	35.6	24.4	0.0 – 66.7
2nd	13	420	25	6.0	15.4	0.0	0.0 – 25.0
3rd	23	82	5	6.1	17.4	17.4	0.0 – 100.0
Total	81	1977	350	17.7			

Table III- General Parasitism rates (%) observed in each the studied farms

Farm	Aciprestes	Granja	Cidrô	S. Luiz	Carvalhas	Total
Number of larva/pupa collected	28	419	82	838	610	1977
Number of larva/pupa parasitized	14	12	9	108	207	349
Parasitism rate (%)	**46.4**	2.9	11.0	12.9	**33.9**	17.7

Table IV- Relative proportion of each parasitoid specie of *Lobesia botrana* identified in this study

Farm	Aciprestes	Granja	Cidrô	S. Luiz	Carvalhas	Total	%
Taxa (Morpho-specie)	**46.4**	2.9	11.0	12.9	**33.9**	17.7	
Campoplex capitator (B)		2		33	5	40	**11.8**
Ichneumonidae (B2)				1		1	0.3
Elachertus affinis (C)	13	5	8	55	193	274	**80.8**
Chalcidoidea (C3)				1		1	0.3
Chalcidoidea (C4)					1	1	0.3
Brachymeria sp. (G)				4	4	8	**2.4**
Elasmus sp. (K)				1	1	2	0.6
Elasmidae (K2)					1	1	0.3
Elasmidae (K3)					1	1	0.3
Ascogaster quadridentada (D)		4		2		6	**1.8**
Bethylidae (Z)				1		1	0.3
Ichneumonidae (M)					1	1	0.3
Tachinidae (Y)		1	1			2	0.6
Abundance (N)	13	12	9	98	207	340	100
Richness (S)	1	4	3	8	8	14	-
SHANNON INDEX (Parasitoids)	0.0	**1.78**	0.50	**1.57**	0.52	-	-
Eveness (Parasitoids)	-	**0.89**	0.32	**0.52**	0.17	-	-

Table V – Total male captures of *Lobesia botrana* (mean ± SE), in mating disruption and control plots and percentage of disorientation of males in mating disruption plots in relation with control plots (except for the second and third generation of 2011, when disorientation was compared with control plots with insecticide treatment (*)).

		2011			2012		
		Nº of plots	Nº capture	% disor.	Nº of plots	Nº capture	% disor.
1st flight	MD	3	3.67±1.45	98.93±0.66	11	5.09±1.07	94.43±1.26
	Control	3	345.00±126.06		4	96.50±14.89	
2nd flight	MD	3	8.33±1.76	95.39±2.15*	11	1.91±0.87	97.80±1.47
	Control		-			120.50±60.24	
	Control w/ insect	3	292.67±58.30		4		
3rd flight	MD	3	4.33±2.33	0.00*	9	0.11±0.11	98.99±1.01
	MD w/insect	-	-	-	2	0.00±0.00	
	Control	-	-	-	3	4.00±7.72	
	Control w/ insect.	3	38.67±21.94	-	1	3.00	

Table VI - Infestation by *Lobesia botrana* in MD and control plots and infestation reduction in MD plots in relation with control plots, during 2011 (infestation is expressed as the number of nests/100 inflorescences in the first generation, and as the percentage of infested grape clusters in the second and third generations and at harvest).

		Nº of observed plots	Infestation [min, máx]	Infestation redution [min, máx]
1st gen	MD	3	60.98±6.12 [52.8, 73.0]	0.00
	Control	4	51.17±24.83 [11.3, 119.5]	
2nd gen	MD	2	24.85±21.40 [3.4, 46.3]	11.49±11.49 [0, 22.99]
	Control w/ insect	3	6.18±1.40 [4.5, 9.0]	
3rd gen	MD	3	2.22±1.00 [0.0, 4.9]	0.00
	Control w/ insect	3	3.02±1.95 [0.0, 8.2]	
Harvest	MD	3	3.76± 1.78[0.0, 8.2]	0.00
	Control w/ insect	3	1.48± 0.94[0.0, 3.9]	

Table VII - Infestation by *Lobesia botrana* in MD and control plots (with (w/ I) or without (w/o I) insecticide) and infestation reduction in MD plots in relation with control plots, during 2012 (infestation is expressed as the number of nests/100 inflorescences in the first generation, and as the percentage of infested grape clusters in the second and third generations and at harvest).

| | | Nº of observed plots | infestation [min, máx] | Infestation reduction | |
				Control w/o I [min, máx]	Control w/ I [min, máx]
1st gen	MD	11	2.02±0.99 [0.0, 7.5]	74.55±12.63 [5.8, 100.0]	-
	Control	4	11.96±6.14 [0.0, 29.0]		
2nd gen	MD	9	2.30±0.85 [0.0, 7.0]	75.42±9.75 [18.8, 100.0]	-
	Control	4	15.88±7.58 [1.7, 36.7]		
3rd gen	MD	7	2.00±1.15 [0.0, 8.0]	64.29±17.98 [0.0, 100.0]	75.00±14.43 [50.0, 100.0]
	MD w/ I	2	6.00±2.00 [4.0, 8.0]	0.00	25.00±25.00 [0.0, 50.0]
	Control w/o I	3	4.67±1.76 [2.0, 8.0]		
	Control w/ I	1	8.00		
Harv	MD	13	7.50±1.97 [1.0, 26.0]	46.00±9.61 [0.0, 91.5]	31.15±9.23 [0.0, 86.3]
	MD w/I	2	2.00±2.00 [0.0, 4.0]	82.36±17.64 [64.7, 100.0]	71.43±28.67 [42.9, 100.0]
	Control w/o I	3	4.40±2.21 [0.0, 7.0]		
	Control w/ I	1	11.34		