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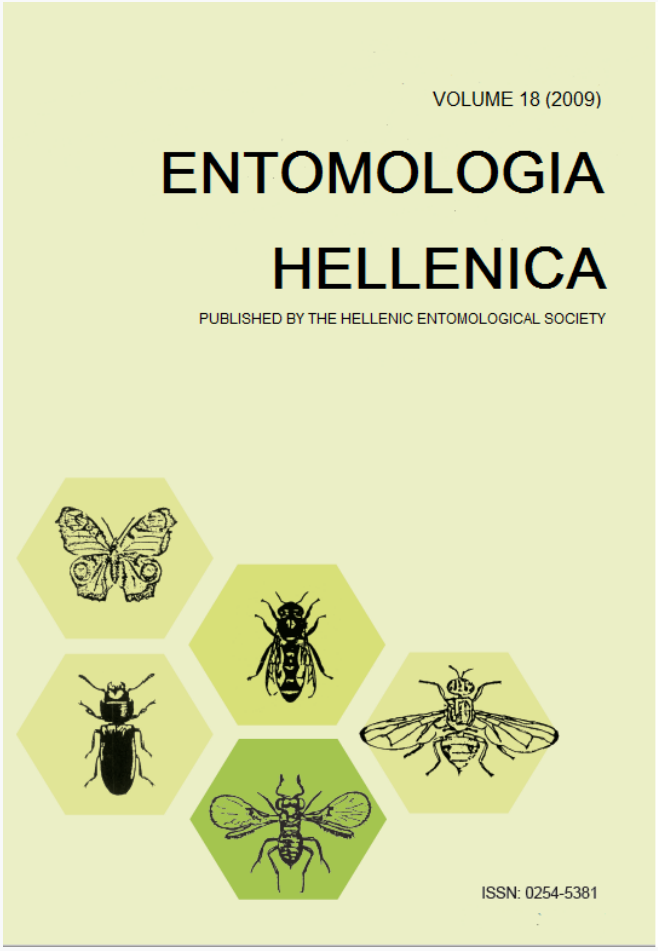

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Efficacy and speed of action of selected plant protection products on *Lymantria dispar* in laboratory conditions

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ABSTRACT

In this study some selected insecticides were evaluated for their effect on gypsy moth *Lymantria dispar* L., (Lepidoptera: Lymantriidae) under laboratory conditions. Diflubenzuron, methoxyfenozide, triflumuron, fenoxycarb, fenoxycarb + lufenuron, *Bacillus thuringiensis* 50% subsp. kurstaki + *Bacillus thuringiensis* 50% subsp. aizawai, *Bacillus thuringiensis* subsp. aizawai and spinosad were used in the recommended concentration, against the 1st, 2nd, 3rd and 4th larval instars of *L. dispar*. The effectiveness of the above insecticides as well as the speed of action ($LTime_{50}$ and $LTime_{90}$) expressed in days, were examined in detail. Spinosad and methoxyfenozide presents a relatively higher speed of action in relation to the other insecticides. $LTime_{50}$ of spinosad and methoxyfenozide did not differ significantly among the first three larval instars and ranged from 0 to 0.61 and 1.13 to 1.74 days, respectively. Regarding IGRs, the mixture (fenoxycarb + lufenuron) and triflumuron were the most effective in relation to the other IGRs tested. Moreover, *Bacillus thuringiensis* toxins were effective only against the first two larval instars.

KEYWORDS: *Lymantria dispar,* methoxyfenozide, spinosad, *Bacillus thuringiensis*, Insect Growth Regulators.

Introduction

The gypsy moth, (*Lymantria dispar* L.), is a serious pest threat of forests, woodlands, shade trees and landscape plants in Europe, Asia, North Africa and the United States (Grijpma 1989). Larvae feed on leaves of forest, shade, ornamental, fruit trees and shrubs but prefer mostly *Quercus* species (Leonard 1981). Severe outbreaks of the gypsy moth are observed since 2005 in the region of Chalkidiki in Makedonia. In the above area the gypsy moth has one generation per year (Markalas and Kalapanida 1999). In vulnerable forest types gypsy moth populations can increase to a point as long as natural enemies no longer exert effective control. Populations then build up within about 3 years to outbreak levels. In peak years of severe outbreaks in

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oak-dominated forests, 100% defoliation of all favored to moderately resistant trees often occurs (Schweitzer 2004). Egg masses are deposited from late August to April, underside of branches, on tree trunks, in stone walls, fences, under eaves of houses, under dead bark of trees, inside birdhouses, under ivy, etc.

Control of *L. dispar* is relatively difficult and the risk of damage from this pest is extremely high every year. So far chemical insecticides have been widely used against gypsy moth populations (Schweitzer 2004). However, the widely use of insecticides is possible to lead to resistance development. Therefore the choice of the most appropriate insecticide is a very important decision (Berrada and Nguyen 1994). Moreover, gypsy moth spraying might completely eradicate localized populations of non-target Lepidoptera (Wagner et al. 1996, Peacock et al. 1998, Severns 2002). Therefore, alternative control tactics as well as insecticides with different mode of action are needed for a successful management of the gypsy moth. A wide range of synthetic broad-spectrum insecticides such as organophosphates, carbamates or pyrethroids are still widely used against this pest in many countries. More selective, insect growth inhibitors (IGIs) and regulators (IGRs) have become increasingly popular in the last years, particularly in Europe (Charmillot et al. 2001). However, few compounds have been evaluated in the field or even in the laboratory for their ability to reduce gypsy moth populations. The extremely high investment needed to market any new insecticide has also led to the use of predictive models in an attempt to reduce the number of insecticides that are retained after initial screening for more detailed assessments (Matthews 1997). The purpose of this study was to evaluate several insecticides for their effect and speed of action (expressed as lethal time) on different larval instars of the gypsy moth, as key information for an efficient control of this pest. Moreover, this data can prove useful in resistance management or in integrated pest management programs. Therefore, certain insecticides belonging to groups with different mode of action were evaluated in the laboratory.

Materials and Methods

Insects

Insects originated from egg masses that were collected from fields of northern Greece located in the prefecture of Chalkidiki (Galatista). Young and fully-grown *Quercus* spp. leaves were collected daily and used as food for larvae of *L. dispar* which were held in plastic cages (55x30x40cm) in an insectary at the Plant Protection Institute of Thessaloniki. Cages were made of ventilated plastic lid with two, across each other, windows (5x20cm) of plastic mesh (Ø 0.1 mm). All experiments were conducted under laboratory conditions at 26±1°C, 55±5% R.H. and under a photoperiod of 15:9 (L:D). Lighting was provided by white fluorescent lamps with light intensity of about 13 watt/m^2.

Effectiveness and speed of action

The effectiveness and speed of action of eight insecticides belonging to groups with different mode of action were evaluated in the laboratory. Those products were:

a) A natural insecticide: spinosad (Laser, Ελάνκο Ελλάς AEBE). It has both contact and stomach activity against lepidopteran larvae, leaf miners, thrips, and termites. Spinosad acts by disrupting binding of acetylcholine in nicotinic acetylcholine receptors at the postsynaptic cell (Salgado et al. 1997).

b) Two biological insecticides: i) 50% *Bacillus thuringiensis* subsp. kurstaki + 50% *Bacillus thuringiensis* subsp. aizawai (Agree, Syngenta Hellas AEBE) and ii)

Bacillus thuringiensis subsp. aizawai (Xentari, BASF Ελλάς ABEE). *Bt* products control most lepidopteran pests, especially larvae with high gut pH, including armyworms, cabbage looper, imported cabbage worm, gypsy moth and spruce budworm (Ware and Whitacre 2004).

c) A moulting hormone agonist insecticide: methoxyfenozide (Runner, Bayer Ελλάς ABEE). Methoxyfenozide is a hydrazine insecticide/IGR (a newer class of insecticidal IGRs) and is considered as a reduced-risk candidate which was first registered by U.S. Environmental Protection Agency in mid 2000 (Ware and Whitacre 2004).

d) An insect growth regulator (IGR), juvenile hormone mimic: fenoxycarb (Insegar, Syngenta Hellas AEBE). Fenoxycarb is a carbamate IGR that has also juvenile hormone type effects when contacted or ingested by a wide array of arthropod pests (Ware and Whitacre 2004).

e) Three insect growth inhibitors (IGI), chitin synthesis inhibitors: i) diflubenzuron (Dimilin, Άλφα Γεωργικά Εφόδια AEBE), ii) triflumuron (Alsystin, Bayer Ελλάς ABEE) and iii) a mixture of fenoxycarb with lufenuron (Lufox, Syngenta Hellas AEBE).

f) For control we used distilled water.

All products used for the efficacy experiment were applied against the 1st, 2nd, 3rd and 4th larval instars of gypsy moth. Separation of larval instars was achieved by selecting insects about 3 days after egg hatching and transporting them into a new separate cage. Eleven days after egg hatching, most of them via a molting passed into the second instar, and were transferred into a new cage. After 12 days second instar larvae passed in the third larval instar and finally third instar larvae passed in the fourth instar in about 15 days. Fourth larval instar lasted 18 days. Larvae of each instar were distinguished into a separate cage.

The application rate (per 100 liters) of each active ingredient of the above eight products was: spinosad (4.8 ml), *B. thuringiensis* subsp kurstaki + aizawai (3.8 g), *B. thuringiensis* subsp aizawai (3 g), methoxyfenozide (28.8 ml), fenoxycarb (20 g), triflumuron (12.5 g), fenoxycarb 7.5% + lufenuron 3% (10.5 ml) and diflubenzuron (15 g). These doses were used against the 1st, 2nd, 3rd and 4th larval instar of gypsy moth. Overall four replicates were conducted of 92, 56, 48 and 48 larvae of the 1st, 2nd, 3rd and 4th instar, respectively. All products as well as the control were applied in topical spray assays with a Potter precision spray tower (Burkard Manufacturing Co. Ltd., Rickmansworth Herts, UK) with 11 cm maximum diameter of specimen dish, 24 ml maximum capacity of reservoir and 2 Kg/cm^2 maximum working pressure. Larvae in treatment groups were sprayed with a spay pressure of 0.5 Kg / cm^2 and with 0.05 ml aqueous solution of the test material / specimen dish (approximately to 200 litter per ha). Afterwards, larvae were individually transferred to clean plastic cages and mortality was checked every day, for 6 days. Every day larvae were provided with fresh food.

Statistical analysis

For the statistical analysis of our data we used probit analysis of correlated data (multiple observations over time at one concentration) (Throne et al. 1995). In a software program (* PROBIT, Jim Throne USDA, ARS, USGMRL 1996) written in Mathematica reg. language by J. Throne which allows probit transformation of the proportion of the insects that were killed, correlated serial time-mortality data were analyzed for every treatment per larval instar of *L. dispar* after correction for control mortality using Abbott's formula (Finney 1957). Six observations at six time intervals

(days) were done for each larval instar treatment.

Results

Effectiveness (proportion of insect killed) and speed of action (lethal time expressed in days) of the insecticides used against the first larval instar of *L. dispar* are presented in Fig.1A except for spinosad as all larvae died within the first 24 h. Approximately 50% of the larval population treated with *B. thuringiensis* subsp. aizawai and triflumuron were killed during the first day. Mean lethal time ($LTime_{50}$) of first instar larvae exposed to *B. thuringiensis* subsp kurstaki + aizawai (1.16 days), methoxyfenozide (1.62 days) and fenoxycarb + lufenuron (1.81 days) were less than 2 days and did not differ significantly. Time needed to cause 90% mortality ($LTime_{90}$) when treated with fenoxycarb + lufenuron, methoxyfenozide and *B. thuringiensis* subsp. aizawai was 3.67, 3.97 and 5.31 days, respectively (Table 1).

Regarding the effectiveness of the insecticides against the 2nd instar larvae, spinosad killed all 2nd instar larvae within the first 24 h (Fig. 1B). $LTime_{50}$ of the larval population treated with fenoxycarb + lufenuron was nearly one day (0.99 days). Relative low $LTime_{50}$ was observed for methoxyfenozide (1.74 days) followed by *B. thuringiensis* subsp kurstaki + aizawai (3.21 days). Mortality of 90% of the 2nd instar larvae treated with methoxyfenozide occurred five days after treatment (Table 1).

In Fig. 2A it is presented mortality of 50% of the 3rd larval instars group treated with spinosad (0.61 days). $LTime_{50}$ of the larval population treated with methoxyfenozide ($LTime_{50}$=1.13) and triflumuron did not differ significantly (1.13 and 1.86 days, respectively). Significantly lower $LTime_{90}$ of the 3rd larval instars population was observed when treated with spinosad (3.30 days) and methoxyfenozide (3.33 days) in relation to the other insecticides (Table 1).

Regarding the 4rth larval instar group, spinosad killed within the first day more than 50% of the larval population (Fig. 2B). Triflumuron needed the lowest time to cause 50% mortality of the 4rth instar larvae followed by methoxyfenozide (1.45 and 3.67 days, respectively). Moreover, 90% mortality of the 4rth larval instars population treated with methoxyfenozide and triflumuron occurred 5.25 and 7.14 days after treatment (Table 1).

Discussion

According to our results, spinosad and methoxyfenozide acted faster against all larval instars. Their effectiveness does not change with the larval instar as far as it is concerned the first three larval instars (L_1, L_2 and L_3). $LTime_{50}$ values for spinosad and methoxyfenozide during the first three larval instars ranged from 0 to 0.61 and from 1.13 to 1.74 days, respectively. Spinosad was more effective until the 3rd larval instar but against the 4th larval instar it needed more than 16 days to cause mortality to 90% of the larvae ($LTime_{90}$), even though 50% of the larval population was killed within the first day.

The mixture fenoxycarb + lufenuron as well as triflumuron were more effective than the other two IGRs (diflubenzuron and fenoxycarb). More specifically, fenoxycarb + lufenuron acted faster against the first two larval instars ($LTime_{50}$ values were 0.99 and 1.81 days, respectively), while triflumuron acted faster against the 3rd and 4th larval instars ($LTime_{50}$ values were 1.86 and 1.45 days, respectively). Regarding diflubenzuron it acted better against the 3rd and 4th larval instars ($LTime_{50}$ were 7.07 and 6.95 days, respectively). Fenoxycarb acted relatively slow against the first three larval

Table 1. Lethal times causing 50 and 90% mortality ($LTime_{50}$ and $LTime_{90}$) with confidence limits (95%) of all larval instars of *L. dispar*.

Active ingredients	$LTime_{50}$*	(C. l. 95%)	$LTime_{90}$*	(C. l. 95%)	Intercept	Slope	Chi square
1st larval instar							
spinosad		**		***			
B. thuringiensis (kurstaki + aizawai)	**1.16**	0.99 - 1.88	**7.71**	6.20 - 9.40	-0.226	0.196	306.4
B. thuringiensis (aizawai)		**	**5.31**	3.20 - 8.63	0.481	0.151	1041.7
methoxyfenozide	**1.62**	1.20 - 1.98	**3.97**	3.44 - 4.72	-0.887	0.547	80.6
fenoxycarb	**8.81**	6.94 - 10.68		***	-0.860	0.098	92.4
fenoxycarb + lufenuron	**1.81**	1.49 - 2.10	**3.67**	3.20 - 4.10	-1.315	0.728	5.0
diflubenzuron	**5.19**	3.26 - 8.02	**10.39**	7.69 - 11.00	-1.280	0.247	10.6
triflumuron	**0.12**	0.00 - 0.88	**6.30**	4.92 - 8.40	-0.024	0.207	195.0
2nd larval instar							
spinosad		**		***	-		-
B. thuringiensis (kurstaki + aizawai)	**3.21**	2.58 - 3.91	**8.34**	7.68 - 10.10	-0.801	0.250	55.9
B. thuringiensis (aizawai)	**5.13**	1.80 - 8.30		***	-0.343	0.067	148.8
methoxyfenozide	**1.74**	0.73 - 2.58	**5.49**	4.45 - 7.22	-0.594	0.342	0.7
fenoxycarb		***		***			
fenoxycarb + lufenuron	**0.99**	0.00 - 3.08	**9.81**	7.05 - 12.00	-0.144	0.145	3.7
diflubenzuron	**5.84**	4.87 - 7.13	**10.08**	8.47 - 12.00	-1.766	0.302	4.7
triflumuron	**4.05**	3.18 - 4.99	**11.08**	9.73 - 12.00	-0.739	0.182	23.2

Table 1 (continued). Lethal times causing 50 and 90% mortality ($LTime_{50}$ and $LTime_{90}$) with confidence limits (95%) of all larval instars of *L. dispar*.

Active ingredients	$LTime_{50}$*	(C. l. 95%)	$LTime_{90}$*	(C. l. 95%)	Intercept	Slope	Chi square
3rd larval instar							
spinosad	**0.61**	0.00 - 1.33	**3.30**	2.50 - 4.07	-0.293	0.477	1.0
B. thuringiensis (kurstaki + aizawai)	**9.19**	6.30 - 11.40		***	-1.338	0.146	21.8
B. thuringiensis (aizawai)	**6.82**	4.15 - 9.79		***	-0.995	0.146	24.9
methoxyfenozide	**1.13**	0.41 - 1.68	**3.33**	2.71 - 4.34	-0.659	0.582	1.5
fenoxycarb	**14.20**	9.39 - 15.00		***	-2.134	0.150	2.1
fenoxycarb + lufenuron	**3.14**	0.00 - 7.44	**11.29**	6.42 - 15.00	-0.494	0.157	9.9
diflubenzuron	**7.07**	6.06 - 8.88	**10.55**	8.77 - 14.59	-2.604	0.368	4.4
triflumuron	**1.86**	0.96 - 3.28	**8.22**	6.34 - 10.48	-0.376	0.201	17.1
4rth larval instar							
spinosad		**	**16.12**	11.43 - 18.00	0.040	0.077	43.2
B. thuringiensis (kurstaki + aizawai)	**16.34**	11.34 - 18.00		***	-0.682	0.042	330.8
B. thuringiensis (aizawai)	**7.58**	4.49 - 10.56	**17.92**	16.90 - 18.00	-0.940	0.124	65.2
methoxyfenozide	**3.67**	2.70 - 4.72	**5.25**	4.31 - 7.62	-2.984	0.813	8.1
fenoxycarb	**10.21**	7.73 - 14.30	**15.59**	11.00 - 18.00	-2.430	0.238	1.2
fenoxycarb + lufenuron	**6.34**	5.33 - 7.45	**10.28**	8.57 - 13.69	-2.063	0.325	6.5
diflubenzuron	**6.95**	5.73 - 9.01	**11.43**	9.28 - 16.17	-1.991	0.286	6.1
triflumuron	**1.45**	0.50 - 2.70	**7.14**	4.98 - 10.18	-0.325	0.225	40.3

* in days ** $LTime_{50}$ could not be calculated as high mortality was observed

*** $LTime_{90}$ could not be calculated as it exceeded the duration of the larval stage

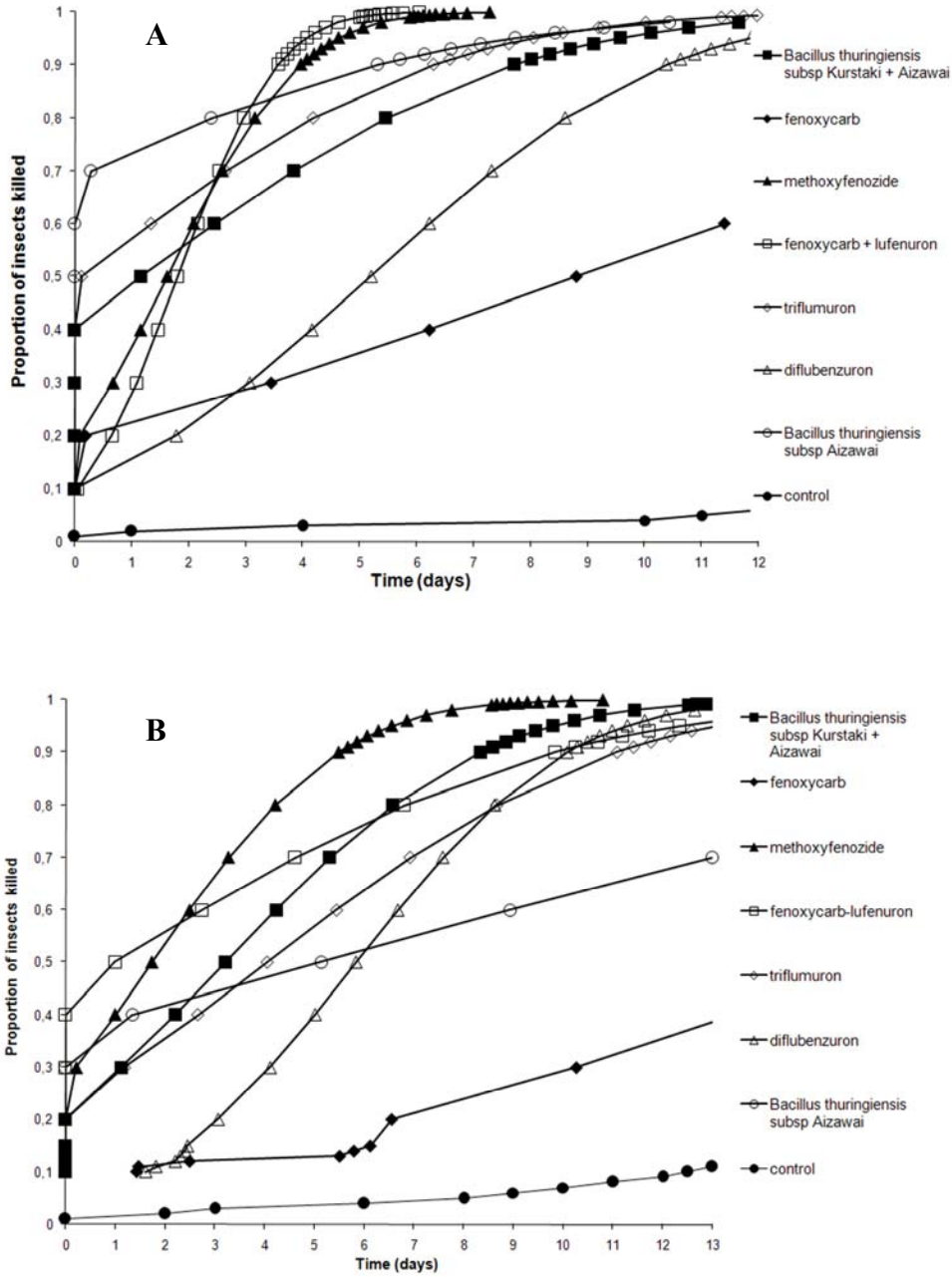

FIG 1. The effectiveness and speed of action of methoxyfenozide, fenoxycarb + lufenuron, triflumuron, diflubenzuron, *B. thuringiensis* subsp. aizawai, *B. thuringiensis* subsp. kurstaki + aizawai and fenoxycarb against A) the 1st larval instar and B) the 2nd larval instar of *Lymantria dispar* under laboratory conditions [26±1°C, 55±5% R.H. and under a photoperiod of 15:9 (L:D)].

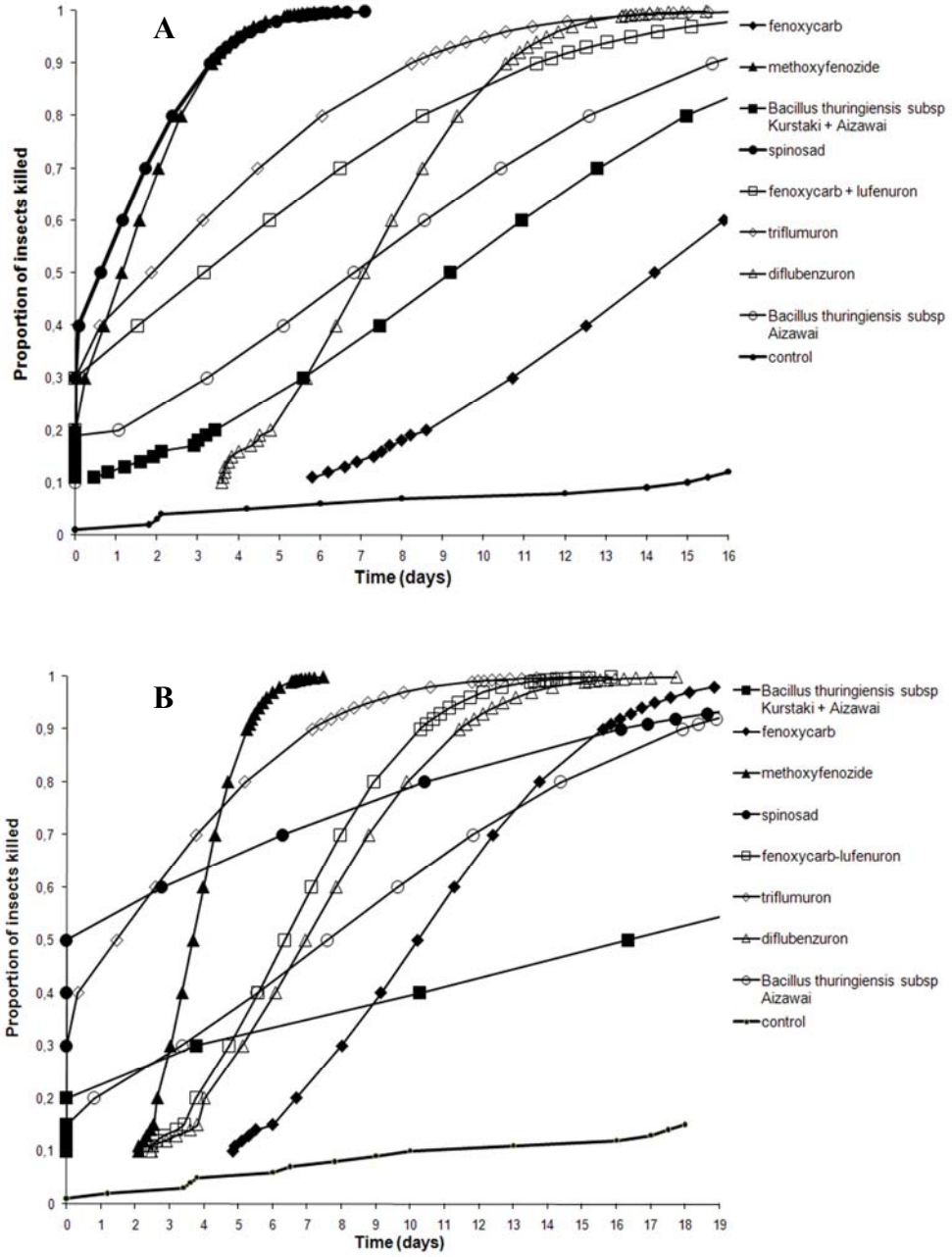

FIG 2. The effectiveness and speed of action of methoxyfenozide, spinosad, fenoxycarb + lufenuron, triflumuron, diflubenzuron, *B. thuringiensis* subsp. aizawai, *B. thuringiensis* subsp. kurstaki + aizawai and fenoxycarb against A) the 3rd larval instar and B) the 4rth larval instar of *Lymantria* under laboratory conditions [26±1°C, 55±5% R.H. and under a photoperiod of 15:9 (L:D)].

instars and does not practically decrease the insect population. In contrast, *B. thuringiensis* products (*B. thuringiensis* subsp kurstaki + aizawai and *B. thuringiensis* subsp aizawai) were more effective against the first two larval instars than against the last two larval instars.

Insect Growth Inhibitors (IGIs) products have both larvicidal and ovicidal properties. Similar mode of action is being observed for IGRs which are analogous to the juvenile hormone, such as fenoxycarb (Dorn *et al.* 1981). Fenoxycarb is known to disturb moulting at the pupal stage and may also have an ovicidal effect. IGR ecdysone agonists, such as methoxyfenozide are known for their larvicidal properties (Charmillot *et al.* 2001).

IGIs and IGRs are very stable and can withstand rain (Charmillot *et al.* 1989). Furthermore, the effectiveness of IGIs and IGRs vary according to the developmental stage of *Platynota idaeusalis* (Lepidoptera: Tortricidae) (Biddinger *et al.* 1998). Thus, complete success in the control of such pests can only be achieved if insecticides are applied in close relation to insect development stage. In terms of its biology and behavior, using IGIs and IGRs as a means to control the gypsy moth can only be effective against the eggs and newly hatched larvae.

Gypsy moth outbreaks as well as most control strategies applied against it have impact on native biota. Even sub-outbreak of the gypsy moth larvae might have impact on native lepidopteran species (Sample *et al*. 1996). In terms of non-target impacts, chemical insecticides have the greatest short-term effect on most native biota of the current management strategies (Schweitzer 2004). The purpose of Integrated Pest Management programs is to monitor insects in the most susceptible stage, as for example happens with spinosad which is more effective against the first two larval instars. However, in order to avoid resistance development the insecticides methoxyfenozide, spinosad and the two IGRs (fenoxycarb + lufenuron and triflumuron) seems to be the most appropriate ones. *B. thuringiensis* toxins were effective only against the first two larval instars, and fenoxycarb only against the 4th larval instar.

Biological control of *L. dispar* can be succeeded only with the use of *B. thuringiensis* toxins one month after the beginning of egg hatching of *L. dispar* because at that time most larvae develop until the 2nd larval instar, which are relative sensitive to *B. thuringiensis* toxins. Primarily, *B. thuringiensis* toxins attack larvae of the order Lepidoptera (Angus 1968). At the same time, beneficial insects are not affected and the natural balance is not disturbed as it happens with long-lasting broad-spectrum insecticides.

Acknowledgements

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Επίδραση επιλεγμένων εντομοκτόνων στο έντομο *Lymantria dispar* σε συνθήκες εργαστηρίου

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ΠΕΡΙΛΗΨΗ

Η *Lymantria dispar* L. είναι ένα πολυφάγο έντομο με κύριο ξενιστή το πουρνάρι (*Quercus coccifera)*. Προσβάλλει διάφορα δασικά πλατύφυλλα και κωνοφόρα είδη, οπωροφόρα και θάμνους. Οι συνέπειες της δράσης του είναι ιδιαίτερα καταστροφικές στο νέο φύλλωμα και στα άνθη, μειώνοντας τόσο την ετήσια αύξηση των δένδρων, όσο και την παραγωγή καρπών των οπωροφόρων. Η δράση του εντόμου επεκτείνεται και στους θάμνους μειώνοντας στο ελάχιστο την διαθέσιμη βοσκήσιμη ύλη. Επίσης έντονα είναι και τα προβλήματα υγείας των κατοίκων της περιοχής που προσβάλλει, αφού το έντομο έχει αλλεργιογόνες ιδιότητες. Στο εργαστήριο δοκιμάστηκε η αποτελεσματικότητα και η ταχύτητα δράσης ορισμένων φυτοπροστατευτικών προϊόντων με διαφορετικό τρόπο δράσης. Οι δοκιμές έγιναν σε προνύμφες πρώτης, δεύτερης, τρίτης και τέταρτης ηλικίας του εντόμου, ξεχωριστά, που προήλθαν από αυγά που συλλέχθηκαν από την ύπαιθρο και εκκολάφθηκαν στο εργαστήριο. Οι δοκιμές αυτές έδειξαν ότι τα methoxyfenozide και spinosad, ακολουθούμενα από τα fenoxycarb + lufenuron και το triflumuron, ήταν πολύ αποτελεσματικά και έδρασαν γρήγορα εναντίον όλων των προνυμφικών ηλικιών του εντόμου που εξετάσθηκαν. Το εντομοπαθογόνο βακτήριο *Bacillus thuringiensis* ήταν δραστικό μόνο εναντίον των δύο πρώτων προνυμφικών ηλικιών και το fenoxycarb μόνο εναντίον της τέταρτης προνυμφικής ηλικίας. Τα αποτελέσματα αυτά δείχνουν ότι σε προγράμματα ελέγχου του εντόμου (με τις πρώτες εκκολάψεις των ωών του εντόμου) θα πρέπει να χρησιμοποιούνται προϊόντα γρήγορης δράσης όπως είναι τα methoxyfenozide και spinosad ή τα fenoxycarb + lufenuron και triflumuron ενώ για την βιολογική καταπολέμηση αυτού του εντόμου (ημιαστικές, περιαστικές περιοχές) θα πρέπει να χρησιμοποιηθεί στον κατάλληλο χρόνο το *B. thuringiensis*, περίπου ένα μήνα μετά τις πρώτες εκκολάψεις όταν δηλαδή το μέγιστο ποσοστό του πληθυσμού του εντόμου είναι στην πρώτη και δεύτερη προνυμφική ηλικία.