Cytological investigations with regard to population dynamics of pine sawfly, Diprion pini L. (Hym.: Diprionidae)

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Monitoring Gypsy Moth (*Lymantria dispar* L.) Populations (Lep., Lymantriidae) in Slovakia using Pheromone Traps

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ABSTRACT This paper presents the use of pheromone traps to monitor gypsy moth populations in Slovakia. This insect has been the most destructive defoliator in Slovakia since 1986. This study involved optimization of pheromone (attractant, trap and dispenser), determination of the minimum number of sample trees and pheromone trap sets required for statistical evaluation, long-term assessment of population densities and gypsy moth males captured by pheromone traps, and specification of relationships between population density and numbers of gypsy moth males captured by pheromone traps (by means of correlation analysis). Based on the results obtained, it is possible to use pheromone monitoring mainly during the latency and ascending phases. However, in some cases, results are not universal. A considerable part of the results may be influenced by inaccuracies, e.g., relatively low sample sizes due to physical feasibility limitations of the study and immigration of males. In the next outbreak cycle of the gypsy moth, the proposed system will be completed and used as a component of the integrated pest management of gypsy moth in Slovakia.

GYPSY MOTH IS the most serious defoliator of broadleaved stands (mainly oak stands) in Slovakia. The outbreak range covers the lowlands in the south of Slovakia (Fig. 1). Outbreaks of this pest are repeated in cycles of 6 to 12 years. During the last outbreak of 1992 - 1994, gypsy moth damaged more than 18,000 ha of forest stands. The majority of these stands are characterized by bad health as a result of long-term oak decline. Defoliation can cause increased tree mortality in subsequent years. Therefore, attacked stands are treated by a biopesticide (B.t. FORAY 48 SC).

There are two reasons to find a new method for monitoring population density:

1) Gypsy moth population densities are measured using the Turcek method in Slovakia. Using this method, it is possible to get accurate data on pest abundance if the population density is higher than 1.0-1.5 egg masses per tree. However, at such population densities, the pest defoliates stands to such an extent before aerial application of biopesticides (B.t. mainly) that the biopesticide might not be captured by the canopy and falls to the forest floor.

2) Applications named "advance control" or "control in advance" are made when pest population densities have not reached an outbreak level yet, but the trend of increasing abundance has begun.

Pages 70-82 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

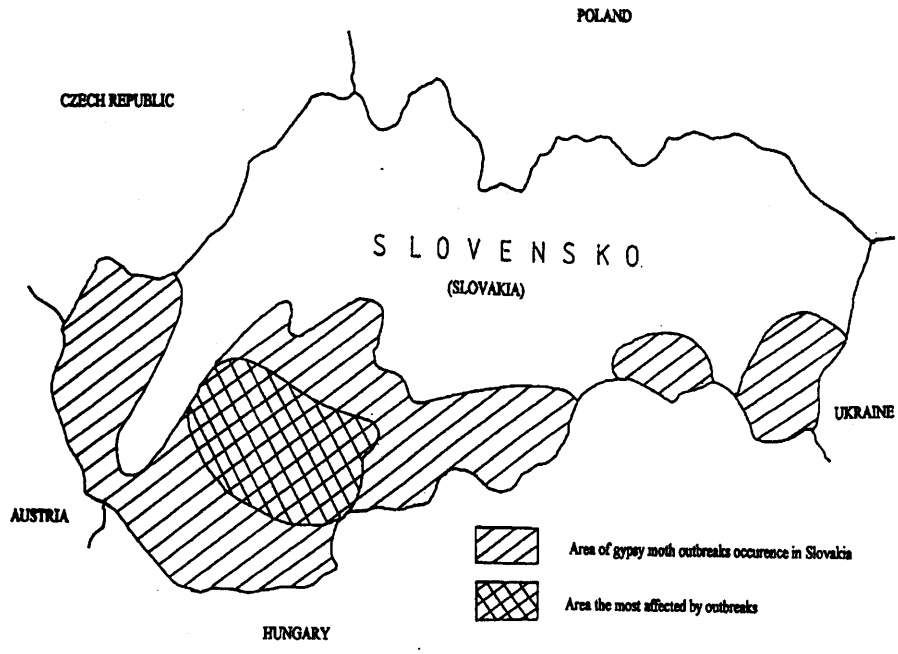

Figure 1. Outbreak area of gypsy moth in Slovakia.

Research on the use of pheromones to study low population densities can be divided into the following steps:

1. Optimization of pheromone (attractant, trap and dispenser);
2. Determination of the minimum number of sample trees and pheromone trap sets required for statistical evaluation;
3. Long-term assessment of population densities and gypsy moth males captured by pheromone traps; and
4. Specification of relationships between population density and numbers of gypsy moth males captured by pheromone traps by means of correlation analysis.

Material and Methods

Pheromone optimization started in 1985. Basic information about field experiments is provided in Tables 1 and 2 and Figure 2. Disparlure in different concentrations was used as an attractant. The types of traps used in the experiments are illustrated in Figure 3. The numbers of gypsy moth males captured at different doses of attractant using different types of traps and dispensers were compared and evaluated statistically.

Criteria for the optimal trapping set (attractant, trap and dispenser) include:

- low variability in numbers of captured males among traps, and
- time efficiency

Table 1. Basic information on the experiments during optimization

Date of experiments	Localities of experiments	Arrangement of testing points	Point spacing	Distance of testing group	Frequency of checking
7/17-9/14	Cifare Tesárske Mlynany	8 components 2 strips	50 m	100 m	14 days
7/15-7/23 7/15-9/3	Èifáre	latin square 9 components, 3 squares	100 m	200 m	every day 14 days
7/27-9/28	Cifare Dolné Semerovce Duzavska lúka	latin square 9 components, 3 squares	50 m		
7/19-9/20	Cifare Bušince Rátka	8 components 2 strips	50 m	100 m	
7/20-9/21	Cifare	latin square 9 components, 2 squares	100 m	200 m	
7/15-8/31	Cifare Rátka Kurinec Nitra Vojnice Levice	5 triangular points in every locality	100 m	minimally 200 m	without checking

In 1990, we tried to estimate the variability of gypsy moth egg masses in a stand. Egg masses were counted on sample trees at different population density levels. We have used the following formula for calculating the minimum sample size of trees and traps.

$$N = \frac{z^2 \cdot s_x^2}{Sypr^2}$$

Where: N = minimum number of samples needed with x% reliability, y% reliability and y% accuracy

z = coefficient of reliability level with P = x%

s_x = coefficient of variation

Sypr = accuracy of assessment

I. • 1 • 2 • 3 II. • 1 • 2 • 3 • 4 III. • 1

• 4 • 5 • 6 • 5 • 6 • 7 • 8 • 2 • 3

• 7 • 8 • 9

Figure 2. Array of testing components in the experiments: I - Latin square, II - Array in strips, III - Triangular array.

Table 2. Data on pheromones, dispensers and traps used in the optimization process

Pheromone trade name	Dose in µg	Dispenser	Attractant	Producer of pheromone and dispenser	Type of trap	Trap producer
NŠ 0,05	50	PO	cis-7,8-epo- 2 Me-18 Hy disparlure (D)	VNIIBMZR Kišinev	DK+1	VNIIBMZR Kišinev
LMS	100	PZ		AK Slušovice		
LM86	100	PZ		CSAV UOCHB Praha	PPT	VÚLHM Jíl. Strn.
LM61	100	KK	D		ÈBL	
LM62	10	KK				VNIIBMZR Kišinev
LM63	1	KK			DK+1 DK-1	
Lymodor	300	PZ		PAN Warszawa	DKO, PL	PAN Warzsawa
					WT	Albany,USA
LMD			D	AK Slušovice	DK+1	VNIIBMZR Kišinev
Etokap	100	KK				
NŠ Kišinev	10	GH		VNIIBMZR Kišinev		
Lymodor	300	PZ		PAN Warzsawa		
LMD			D		DI	SAV ÚEFE
Etokap	100	KK		AK Slušovice		
					HI	LVÚ VS B. Štiavnica
					DK+1	VNIIBMZR Kišinev
LMD					DK+1	VNIIBMZR Kišinev
Etokap	100	KK	D	AK Slušovice		
					PK	AK Slušovice
LMD					DK+1	VNIIBMZR Kišinev
Etokap	100	KK	D	AK Slušovice		

[1]Dispensers: GH - Rubber hose, KK - Rubber conical cup, PO - Plastic rectangular dispenser, PZ - Penicillin plug

Traps: CBL = Czech barrier lantern (dry), DI = Disc (dry, insecticidal), DK + I = Delta Kishinyev with window (dry, insecticidal), DK - I = Delta Kishinyev with window (without insecticide, dry), DKO = Delta Kishinyev open (sticky), HI = Mushroom (dry + insecticide), PK = Plastic container (dry), PL = Polish lantern (dry), PPT = Zinc - coated sheet (sticky), WT = Wing Trap (sticky)

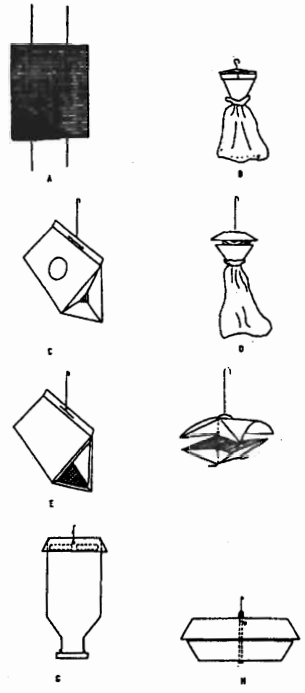

Figure 3. Types of traps used in testing experiments.

A - Zinc coated sheet (sticky)
B - Polish lantern (dry)
C - Delta Kishinyev + insecticide (dry)
D - Czech barrier lantern (dry)
E - Delta Kishinyev open (sticky)
F - Wing trap (sticky)
G - Mushroom + insecticide (dry)
H - Disk + insecticide (dry)

Since 1991, the population density has been measured on fixed plots. Pheromone traps have been installed in the same plots since 1991 (Fig. 5).

From 1991 - 1995, we have graphically compared the mean egg mass number per tree and mean number of males captured per trap.

We conducted a correlation analysis to determine relationships (coefficient of determination) between mean population densities obtained by means of the traditional method and mean number of gypsy moth males captured. We conducted a correlation analysis for every year and all localities as well as for each locality across all years.

Two methods were applied. In the first method "a" data on catches were correlated with the arrangement of traps in the experimental plot. In the second method "b" the data from four traps placed in the internal space of the experimental plot were used (Fig. 4).

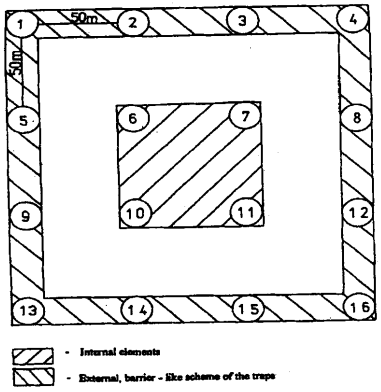

Figure 4. Scheme of pheromone traps on monitoring point.

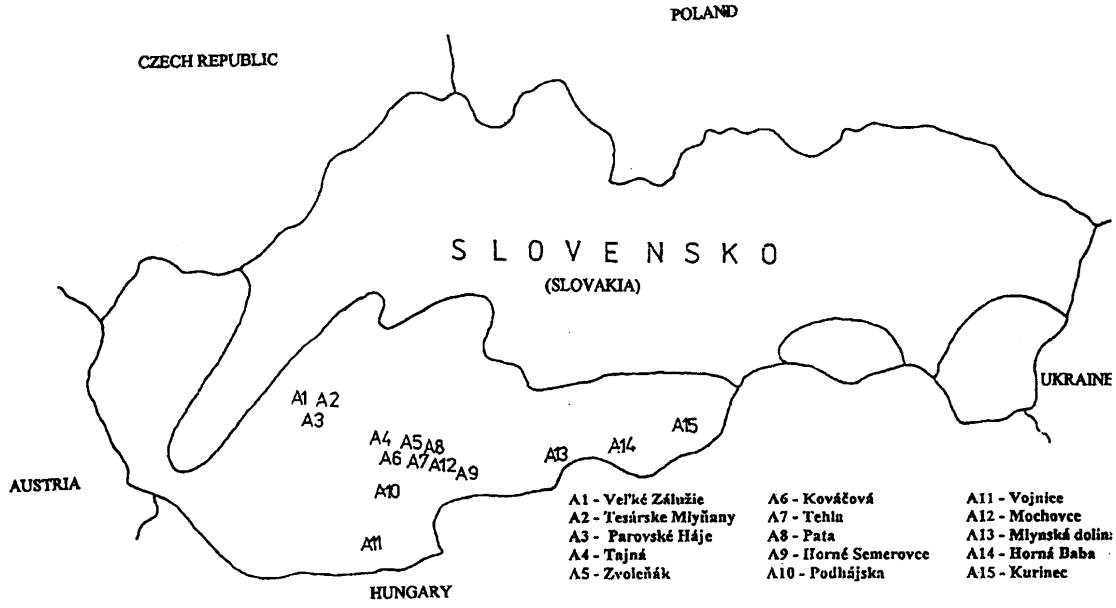

Figure 5. Scheme of experimental plots for monitoring gypsy moth population density.

Results

Pheromone, Dispenser and Trap Optimization. The optimal trapping set that has been used for the last 6 years is the: Pheromone LMD ETOKAP with 1OO g disparlure. The pheromone is formulated in a rubber conical dispenser. The trap is a Delta Kishinyev trap with window + insecticide.

Determination of the Minimum Number of Sample Trees and Pheromone Traps Required. As indicated in Table 3, when population densities are low (below 0.2) a large sample of trees is necessary (more than 300). We obtained a large minimum number of pheromone traps as well (Table 4).

Based on data collected, we determined that the minimum number of sample trees needed is 480 (30 trees in 16 points). For pheromone monitoring, a Latin square (16 pheromone traps with 50 m spacing) was established. The experimental design is presented in Figure 4.

Table 3. Minimum number of trees needed for detection of population density with different measurement accuracies

Population density (Egg masses/ tree)	Selected accuracy 60%	70%	80%	90%
1.00	52	92	207	830
0.40	100	178	400	1,599
0.30	125	222	439	1,997
0.20	171	304	658	2,739
0.10	306	344	1,224	4,896
0.05	560	996	2,241	8,962
0.01	2,454	4,363	9,816	39,266

Table 4. Minimum number of pheromone traps needed for statistical data processing.

Population density (Egg masses/ tree)	Selected accuracy 60%	70%	80%
0.00	5	9	20
0.01	12	21	46
0.05	12	21	46
0.10	6	11	24
0.20	2	3	5
0.40	8	15	33

Long-term Assessment of Population Densities and Gypsy Moth Males Captured by Pheromone Traps. Changes in population density and number of males captured by traps are presented in Figures 6, 7, 8, 9 and 10.

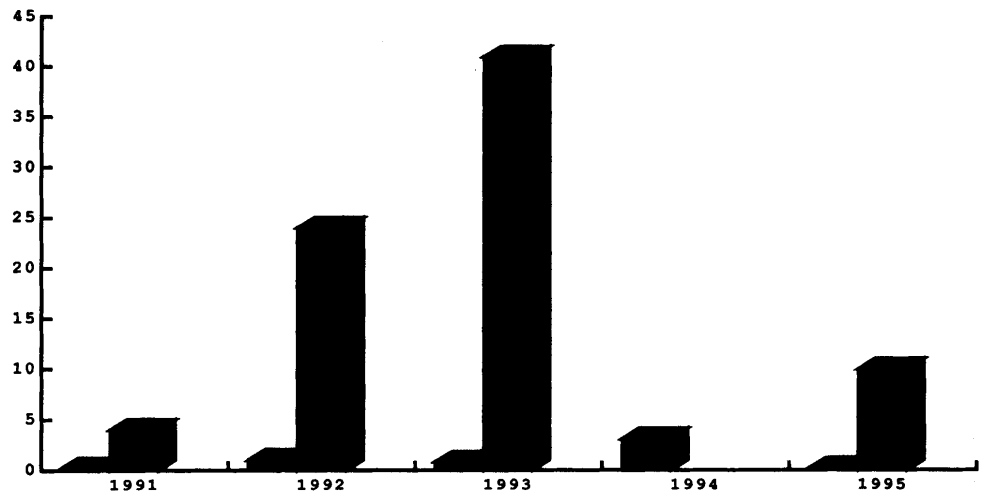

Figure 6. Comparison of mean population density (darker columns) and mean catch of gypsy moth males (lighter columns) in pheromone traps (locality Bušince I). X - axis = years; Y - axis = egg masses / 10 trees and mean number of gypsy moth males captured by 1 pheromone trap.

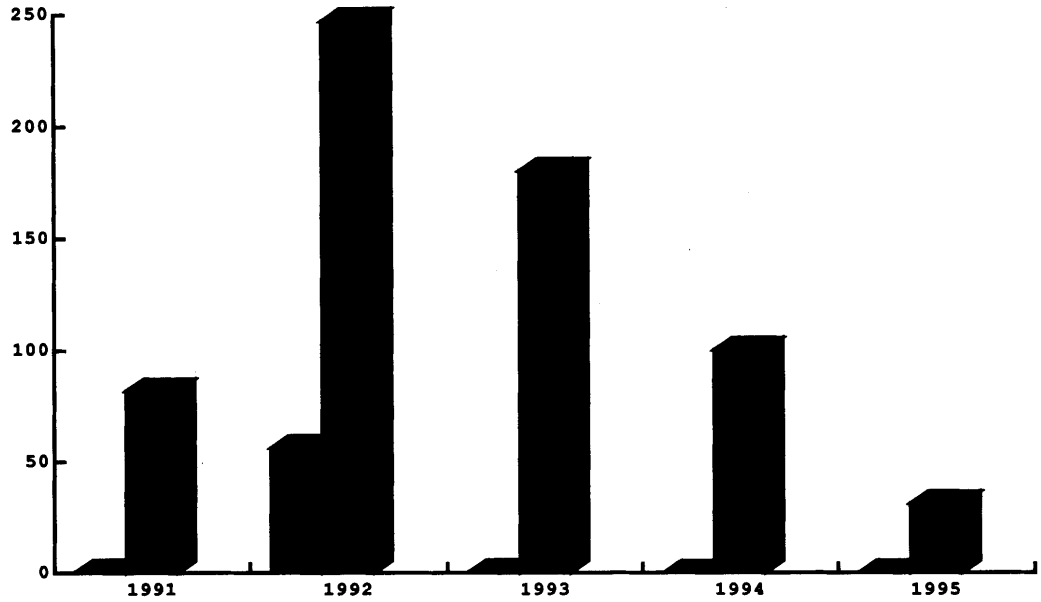

Figure 7. Comparison of mean population density (darker columns) and mean catch of gypsy moth males (lighter columns) in pheromone traps (locality Pata). X - axis = years; Y - axis = egg masses/10 trees and mean number of gypsy moth males captured by 1 pheromone trap.

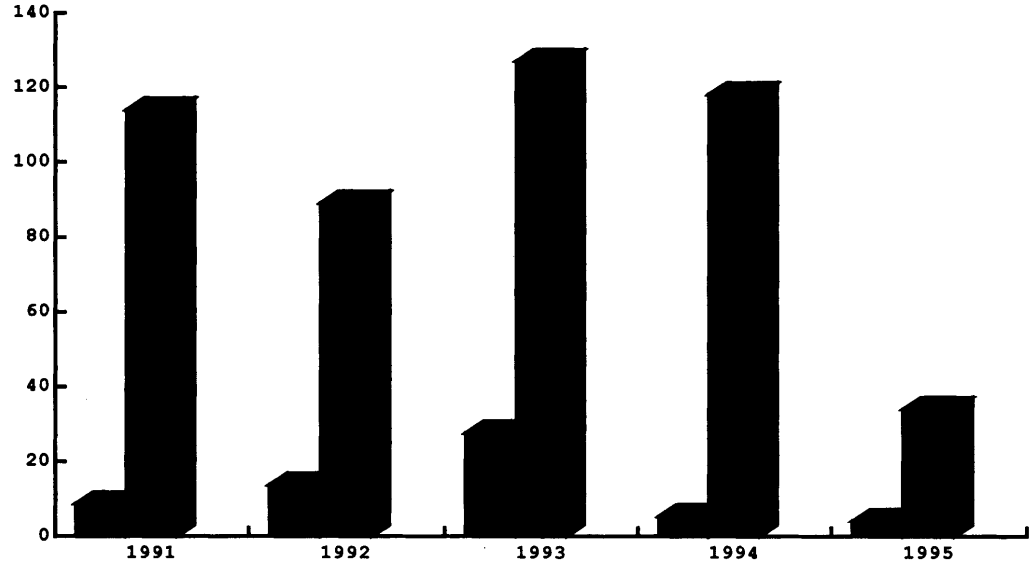

Figure 8. Comparison of mean population density (darker columns) and mean catch of gypsy moth males (lighter columns) in pheromone traps (locality Kovacova). X - axis = years; Y - axis = egg masses/100 trees and mean number of gypsy moth males captured by 1 pheromone trap.

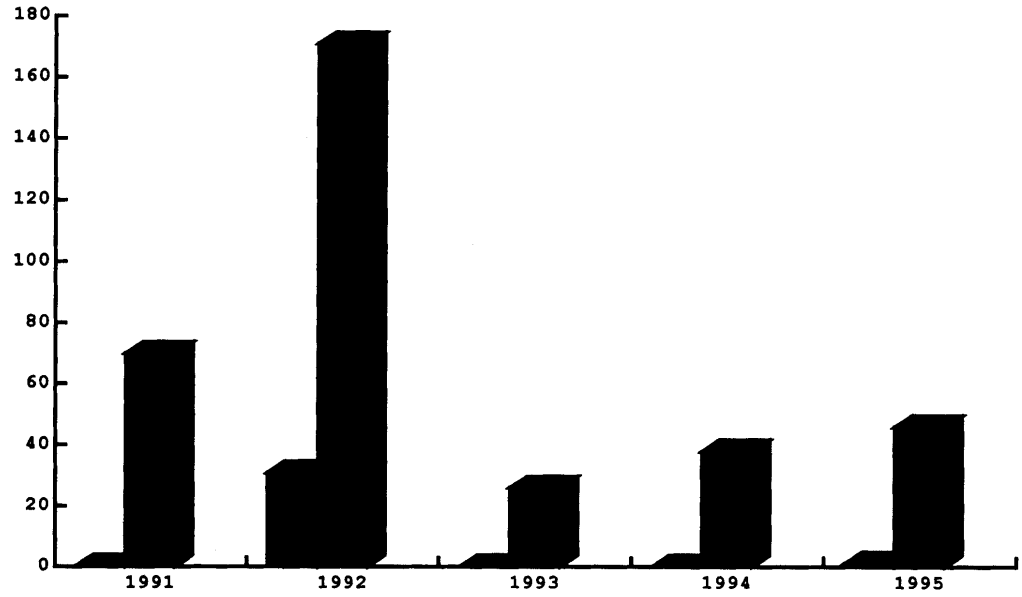

Figure 9. Comparison of mean population density (darker columns) and mean catch of gypsy moth males (lighter columns) in pheromone traps (locality Mlynany). X - axis = years; Y - axis = egg masses/1 tree and mean number of gypsy moth males captured by 1 pheromone trap.

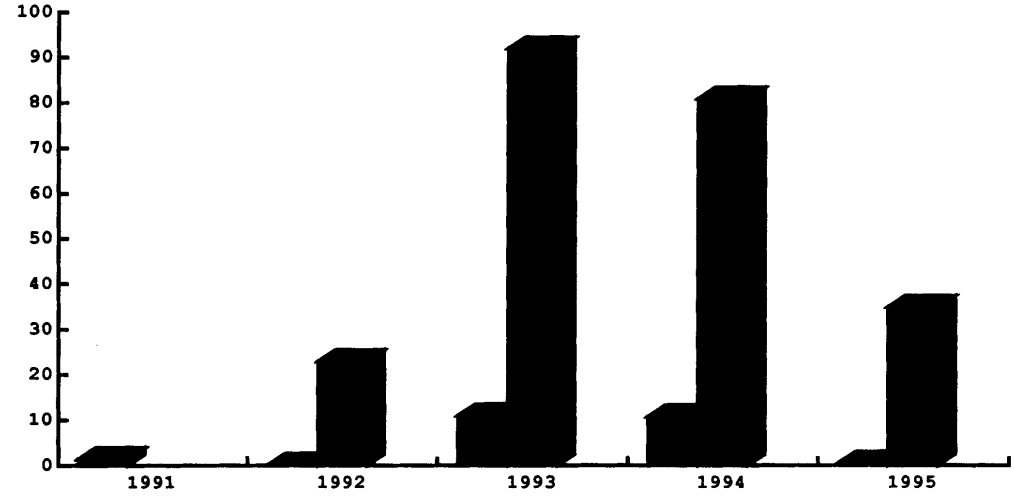

Figure 10. Comparison of mean population density (darker columns) and mean catch of gypsy moth males (lighter columns) in pheromone traps (locality Kurinec). X - axis = years; Y - axis = egg masses/100 trees and mean number of gypsy moth males captured by 1 pheromone trap.

It is possible to conclude that population density trends measured by traditional methods correspond to some extent with the catches of gypsy moth males in pheromone traps, mainly in the latency phase period and in the beginning of an outbreak.

Specification of Relationships Between Population Density and Catch of Gypsy Moth Males Captured by Pheromone Traps - Correlation Analysis. In 1991, we used this technique to calculate a coefficient of determination (R^2) of 0.24 for the "a" variant and 0.79 for the "b" variant (Fig. 11). The first value indicates a poor correlation, while the second one indicates a high correlation. In 1992, the coefficient of determination was 0.26 for "a" variant and 0.42 for "b" variant. Hence, the correlation in 1991 was not very high (Fig. 11).

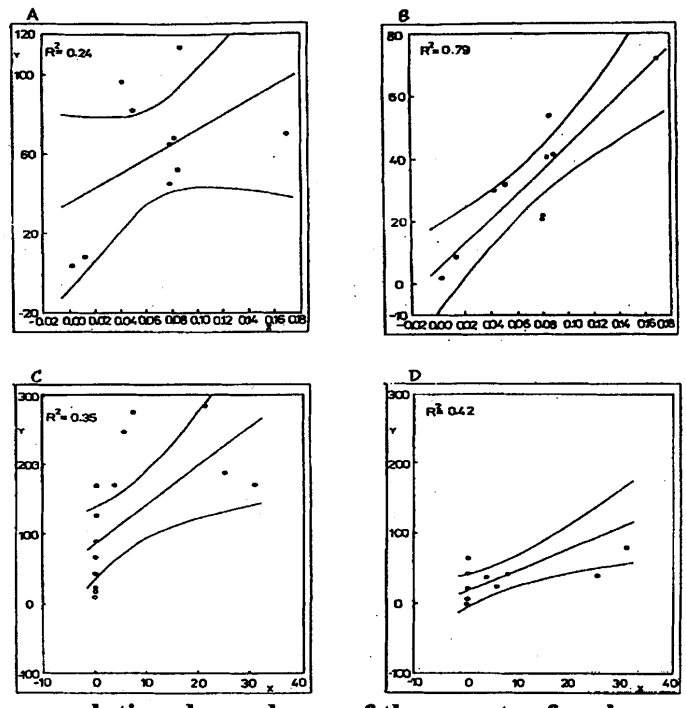

Figure 11. Linear correlation dependence of the counts of males captured in pheromone traps and population density.
A-year 1991 all traps B-year 1991 internal traps
C-year 1992 all traps D-year 1992 internal traps
(x-axis = mean number of egg masses per tree)
(y-axis = mean number of gypys moth males per trap)

The situation in 1993 was entirely different. It can be documented by the results for both variants (a: CD = 0.03, b: CD = 0.002), which indicated an absence of any correlation (Fig. 12). In 1994, the CD reached a value of 0.26 for the "a" variant which is judged as a poor correlation (Fig. 12). In 1995, we recorded 0.47 for the "a" variant and 0.38 for the "b" variant; the correlation was judged as not very high (Fig 13). For individual localities across all years, the R^2 was generally higher than corresponding values for all localities together in the same year. (Tesárske Mlynany = 0.93 from 1991 - 1995, Pata = 0.61 from 1991 -1995, Zvolenak = 0.90 from 1991-1994, and Kurinec = 0.98 from 1992-1994). These data are illustrated by Figure 14.

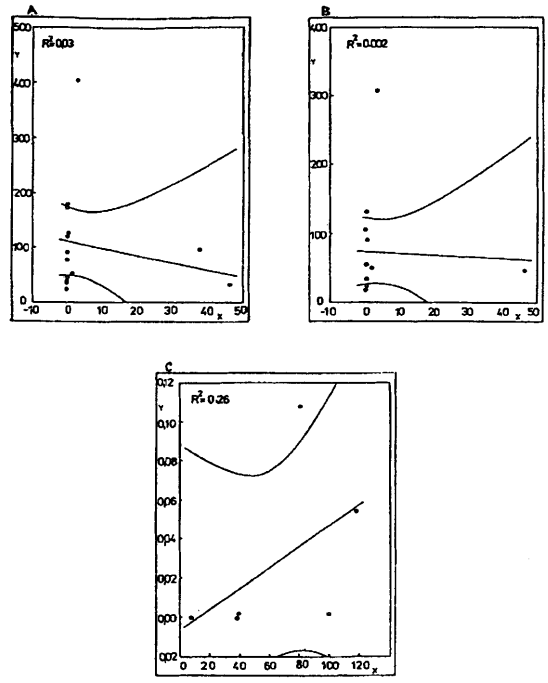

Figure 12. Linear correlation dependence of the counts of males captured in pheromone traps and population density.

A-year 1993 all traps B-year 1993 internal traps
(x-axis = mean number of egg masses per tree)
(y-axis = mean number of gypsy moth males per tree)
C-year 1994 all traps
(x-axis = mean number of gypsy moth males per trap)
(y-axis = mean number of egg masses per trap)

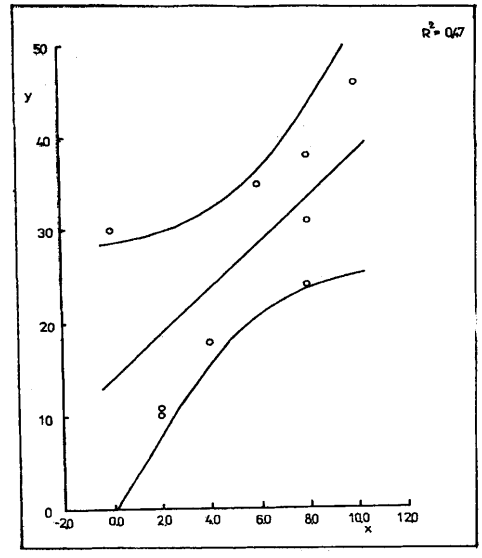

Figure13. Linear correlation dependence of the counts of males captured in pheromone traps and population density (year 1995).

(x-axis = mean number of egg masses per tree)
(y-axis = mean number of gypsy moth males per trap)

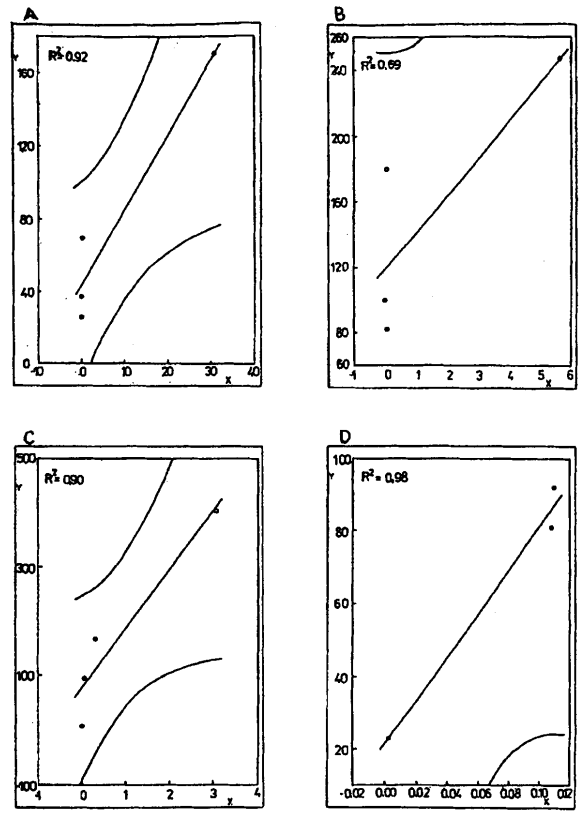

Figure 14. Linear correlation dependence of the counts of males captured in pheromone traps and population density (Period 1991 - 1994.)
A-study plot Tesarske Mlynany B-study plot Pata
C-study plot Zvolenak D-study plot Kurinec
(x-axis = mean number of egg masses per tree)
(y-axis = mean number of gypsy moth males per trap)

Conclusion

Based on the results obtained, the applicability of pheromones depends on the outbreak phases. In Slovakia, which represents the margin of a outbreak area, it is possible to use pheromone monitoring during the latency and ascendency phases. In some cases, results suggest a close correlation between population density and gypsy moth catches in traps. But these results are not universal. A considerable part of the results may be influenced by inaccuracies, e.g., relatively low sample sizes due to physical feasibility limitations of the study and immigration of males.

In Slovakia, the outbreak area of gypsy moth is divided by non-forest areas into many isolated forest areas and thus results should not be substantially affected by male moth migration. Pheromone monitoring can be used as a suitable, complementary method for investigating and predicting population density. Whole design of the application of particular methods during the gradation cycle is illustrated by Figure 15. In the future, the following measures should be implemented to improve pheromone-based monitoring:

- include other methods of data collection for low population densities to compare them with catches in pheromone traps (jute sacks, frass sheets...);
- complete the study of the variability of egg mass occurrence, determining the final set of sample trees needed;
- test new types of attractants, traps and dispensers which would reduce variability of catches;
- field test the proposed monitoring system during an outbreak cycle under field conditions in Slovakia;
- elaborate the standard design of pheromone monitoring for main gradation areas;
- verify correlations between trap catches and population densities in large and closed forest complexes;
- test the barrier effects of pheromone traps; and
- verify time limited monitoring (installing traps for 1 day - 1 month during the flight period) to eliminate the effect of male moth migration from surrounding stands.

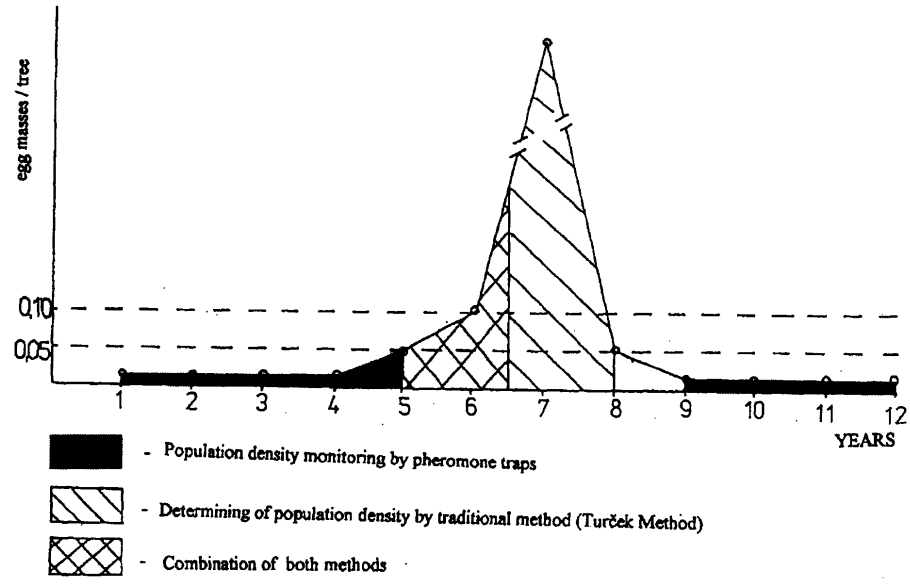

Figure 15. The scheme of the possibilities of methods use for detecting gypsy moth population density during gradation cycle in Slovakia.

In the following gradation cycle of the gypsy moth, the proposed system will be completed and, including recently gained knowledge, it will be used as a component of integrated pest management of gypsy moth in Slovakia.

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Cytological Investigations with Regard to Population Dynamics of the Pine Sawfly, *Diprion pini* L. (Hym.: Diprionidae)

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ABSTRACT The occurrence of diploid males is demonstrated in the pine sawfly, *Diprion pini*. Inbreeding experiments and cytological investigations were designed to investigate this phenomenon. The cytological study shows an unexpected chromosome complement (n=14, 2n=28) in this diprionid species and suggests a karyotype evolution by centric fission. The expected significance of diploid males to *D. pini* population dynamics is discussed.

DIPRION PINI IS an arrhenotokous sawfly whose gregarious larvae defoliate Scots pine, *Pinus silvestris*. In France, long periods of low population level are punctuated by short and spectacular outbreaks. These outbreaks cause considerable damage, reducing the growth of the trees and sometimes leading to tree mortality. *Diprion pini* is consequently responsible for occasional extensive economic loss.

Geographic variation in environmental conditions often results in variation in life cycles and in diapause intensity (Géri and Goussard 1988, 1991). Furthermore, diapause, parasitism and foliage availability (in quantity and quality) are significant regulative factors of population level during outbreaks and their collapses (Géri 1988). However, we cannot explain what triggers outbreaks.

Never the less, several experimental observations suggest an effect of inbreeding on sex-ratio and on diapause rates (Beaudoin et al. 1994, Géri et al. 1995). They suggest the influence of genetic factors. A male-biased sex ratio was notably observed in the rearing strain and following sibmatings while a female-biased sex ratio was generally observed in the field (Beaudoin et al. 1994, Géri et al. 1995). In natural populations, the variations in consanguinity and maybe consequently in sex ratio can affect the population dynamics because the females constitute the demographic potential.

Several mechanisms of sex determination may affect the sex ratio. It is well accepted that hymenoptera reproduction is mostly based on arrhenotokous parthenogenesis: females develop from fertilized eggs and are diploid, whereas males develop from unfertilized eggs and are haploid. In fact, sex determination is not merely a haplo-diploid determination at the cytogenetical level. Whiting (1943) proposed that sex is determined by a series of multiple complementary alleles of one locus (Fig. 1a). Diploid individuals possessing two different sex alleles are females, whereas hemizygous as well as homozygous individuals are males.

Pages 83-90 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

Crozier (1971) extended this scheme to several loci (Fig. 1b). Complementary Sex Determination (CSD) yields several testable predictions. A simple qualitative prediction is that inbreeding should increase diploid male production because it increases homozygosity. Furthermore, the use of controlled matings between relatives permits quantitative predictions. Using mainly inbred crosses, diploid males have been detected to date in about 35 species (Cook 1993b). In some other cases, the sex ratio due to the primary sex determination can be modified by parasitic factors, microorganisms or behavioural control of fertilization. In the parasitic wasp *Nasiona vitripennis,* a supernumerary chromosome causes the condensation of the paternal genome in fertilized eggs. Consequently, the offspring are only haploid males (Beukeboom and Werren 1993).

It's now well known that diploid males have important consequences for population biology in Hymenoptera. In the fire ant *Solenopsis invicta,* diploid males significantly contribute to mortality because they reduce the rate of growth of new colonies and lead consequently to single-queen colony mortality (Ross and Fletcher 1986, 1993). In the parasitic wasp *Diadromus pulchellus*, diploid males modulate the variations in sex ratio due to larval competition in the host (El Agoze et al. 1986). Furthermore, diploid males are increasingly considered as a factor of failure in biological controls using parasitoids (Cook 1993a, Stouthamer et al. 1992).

Several facts assume the occurrence of diploid males in *Diprion pini* and call for cytological investigations to reveal them:

- The single locus CSD is considered ancestral and CSD is clearly widespread in Hymenoptera (Cook 1993b).
- A cytological study (Smith and Wallace 1971) revealed the occurrence of diploid males in another species of Diprioninae, *Neodiprion nigroscutum.*
- In *Diprion pini*, diploid males could account for the sex ratio inversion following inbreeding.
- Heterozygous males have been detected by enzymatic electrophoresis (Beaudoin et al. 1994).

This paper includes the preliminary description of *D. pini* chromosome complement and the results of research on diploid males in this species.

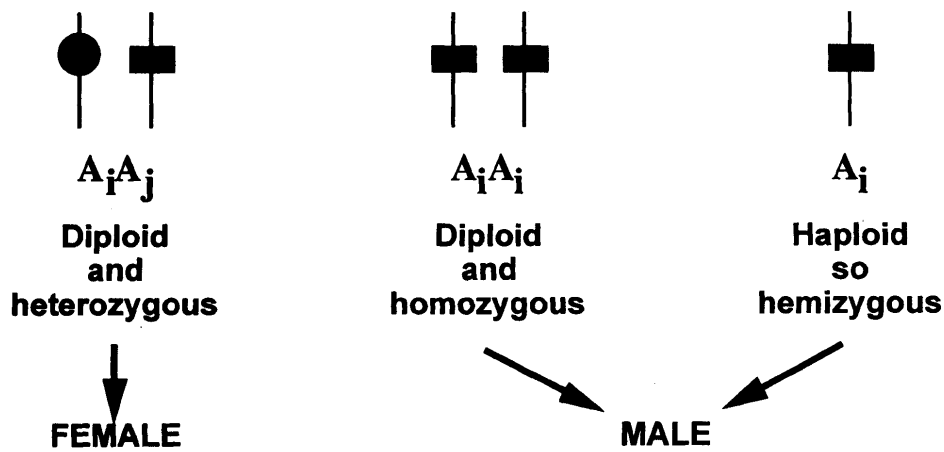

Figure 1a. Single locus CSD; 1 locus, n alleles.

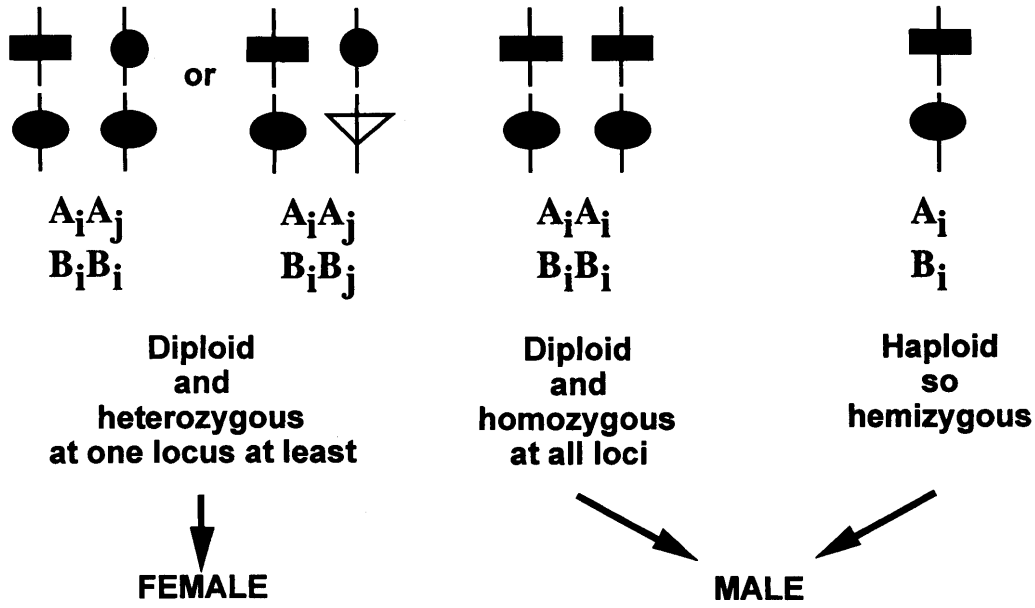

Figure 1b. Multilocus CSD (example of two loci).

Materials and Methods

Under CSD models, well-defined proportions of diploid males are expected following crosses between relatives. Our objective was to produce diploid males by inbreeding and to reveal them cytologically. The main part of the cytological study consisted of finding a tissue at the right time in order to observe as much mitosis as possible. It was necessary to observe many metaphase spreads, because several artifacts are possible, such as a juxtaposition of two chromosome complements that can look like a diploid set.

A. Cytology

Materials. Individuals used in this study were from a rearing strain (native to Sologne, France) or came from France (Fontainebleau Forest and Alps), Finland and England.

Chromosome Preparations. The chromosome number counts were made from the gonads of young prepupae. The gonial mitosis at the beginning of gametogenesis enables the observation of many metaphase spreads without polyploidy problems. We have parallel gametogenesis with eye development, which is a good external marker for dissecting at the right time. Testicles or ovaries were treated in colchicine-PBS solution, then in hypotonic solution for 15 minutes before fixing in an ethanol-acetic acid mixture for 30 minutes. The fixed tissue was then transferred to a drop of 60% acetic acid on a clean slide. Finally, the slide was placed on a warm hotplate and the drop was allowed to evaporate. The preparation was finally stained in a 3% Giemsa solution. Karyotyping followed the standard procedure and centromere position was described according to the nomenclature of Levan et al. (1964).

***In Situ* Hybridization (ISH).** The ribosomal probe used was Py12 cloned in pBr 322. It contains the 18S, 5S, and 28S genes plus intergenic spacers of *Drosophila melanogaster*. Methods for pretreatment, ISH and probe detection followed the procedure described in Engels et al. (1986) with minor modifications.

B. Sibmatings

We performed single-pair matings. Under single-locus CSD, a sister has one chance out of two to mate with one of its brothers possessing one of its alleles, so two kinds of brother-sister crosses occur (Fig. 2). If the female and the male share the same sex allele, their progeny contains diploid males. Consequently, we expected 50% of the colonies to have diploid males. Furthermore, 50% of fertilized eggs in these colonies are expected to be diploid males. Under the two-locus model, we expected 25% of the broods to contain 25% diploid males.

In these experiments, the colonies used for sibmating were descended from mated females collected in the Fontainebleau Forest.

first crosses (in order to obtain brothers and sisters) :

P : **F1 :**

AiAj x Ak → AiAk + AjAk + Ai +Aj

brother-sister crosses (in order to obtain diploid males) :

F2 :

<u>Ai</u>Ak x <u>Ai</u> → <u>AiAi</u> + AkAi +Ai + Ak 50% of diploid males (within the diploid offspring)

AiAk x Aj → AiAj + AkAj +Ai + Ak no diploid males

<u>Aj</u>Ak x <u>Aj</u> → <u>AjAj</u> + AkAj +Aj + Ak 50% of diploid males (within the diploid offspring)

AjAk x Ai → AjAi + AkAi +Aj + Ak no diploid males

Figure 2. Expected proportions of diploid males under a single-locus model.

Results and Discussion

A. Cytology

Our results contradict the study by Maxwell (1958) who karyotyped several samples from an English population of *D. pini*. She described it with a chromosome set of 7. At the beginning of this work, only the males were karyotyped and all had 14 chromosomes. According to Maxwell's description, all the males would be diploid, but our study revealed that all the females have 28 chromosomes. Several populations (see Materials and Methods) were studied, including an English population. All the populations share the same karyotype. So, Maxwell's determinations were not correct concerning *Diprion pini.* In a parallel example, *Diprion similis* was described with a haploid complement of 7 chromosomes by Maxwell (1958) and of 14 chromosomes by Smith (1941, 1960). These discrepancies forced us to redescribe *D. pini* karyotype as a prerequisite.

The 14 chromosomes of the haploid complement are acrocentric. A small chromosome carries a dot-shaped segment which appears similar to a B-chromosome. It is not a supernumerary chromosome but a satellite that is always present once in haploid males and twice in diploid females.

The chromosome number in *D. pini* is twice the basic number in Diprionidae. Two simple hypotheses can be considered to explain this karyotype evolution: polyploidization or centric fission. *In Situ* Hybridization (ISH) was carried out in order to differentiate between haplo-diploidy and diplo-tetraploidy. The rDNA probe hybridizes once in males and twice in females. Haplo-diploidy and centric fission are consequently more likely than diplo-

tetraploidy and polyploidization. This result is interesting from the fundamental and applied points of view (repercussions on sex determination and diploid male production).

B. Sibmatings and Detection of Diploid Males by Means of Cytology

Two series of sibmatings were carried out. Each time, some diploid males were revealed by karyotyping.

Diploid Male Characteristics. In the first experiment, all the individuals were weighed, dissected and karyotyped. Only four colonies (F2) were obtained from fertilized females (F1) and these colonies were not descended from the same "grandmother" (P). Only one progeny contained diploid males. In this colony, 40% of diploid individuals were male. The diploid males were clearly heavier than haploid males (33% on average), but the weight distributions slightly overlap each other (Fig. 3). Diploid males were responsible for sex ratio inversion. Because of homozygosity at the sex locus (or loci), the proportion of males at the prepupae instar was 56% while it would have been 27% if all the fertilized eggs developed into females.

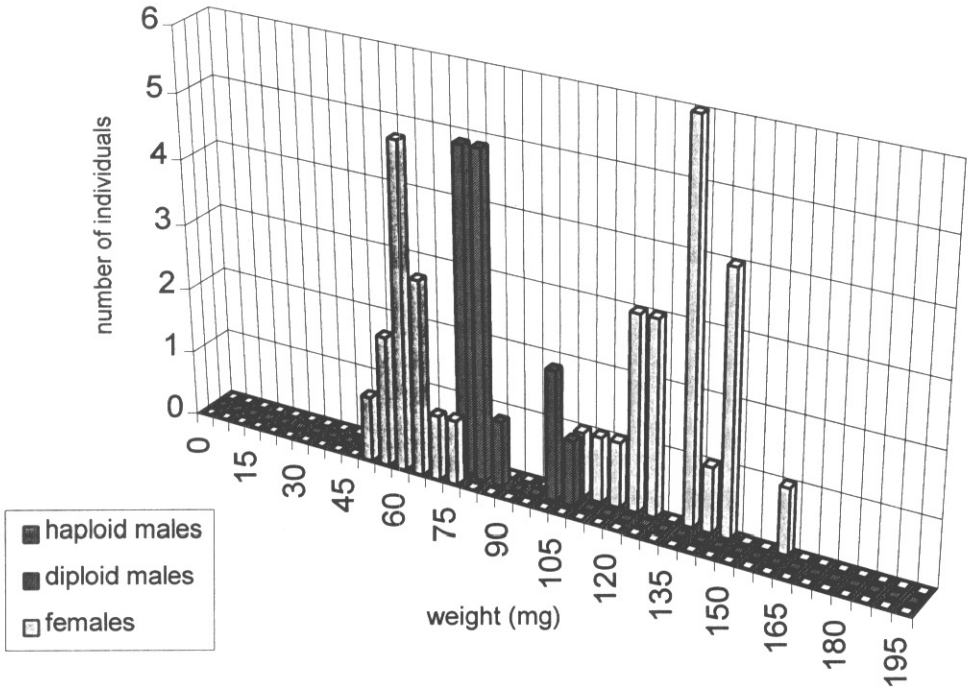

Figure 3. Weight distribution in a colony containing diploid males.

A second experiment was carried out in order to obtain diploid male adults. Because the weight of haploid male cocoons is rarely higher than 90 mg, individuals were graded by weight: < 90 mg and ≥ 90 mg. In this experiment, only a sample was dissected and karyotyped. All the dissected males among the heaviest males were diploid. Consequently, all the non-dissected males in this range were assumed to be diploid. So, it was confirmed that diploid male prepupae were heavier than haploid male prepupae (Fig. 4). The presumed diploid male imagos as a whole were clearly heavier and bigger with regard to many morphological features, but it was not possible to discriminate between a haploid male and a diploid male. Each time, the distributions overlapped and no typical marker was found until now.

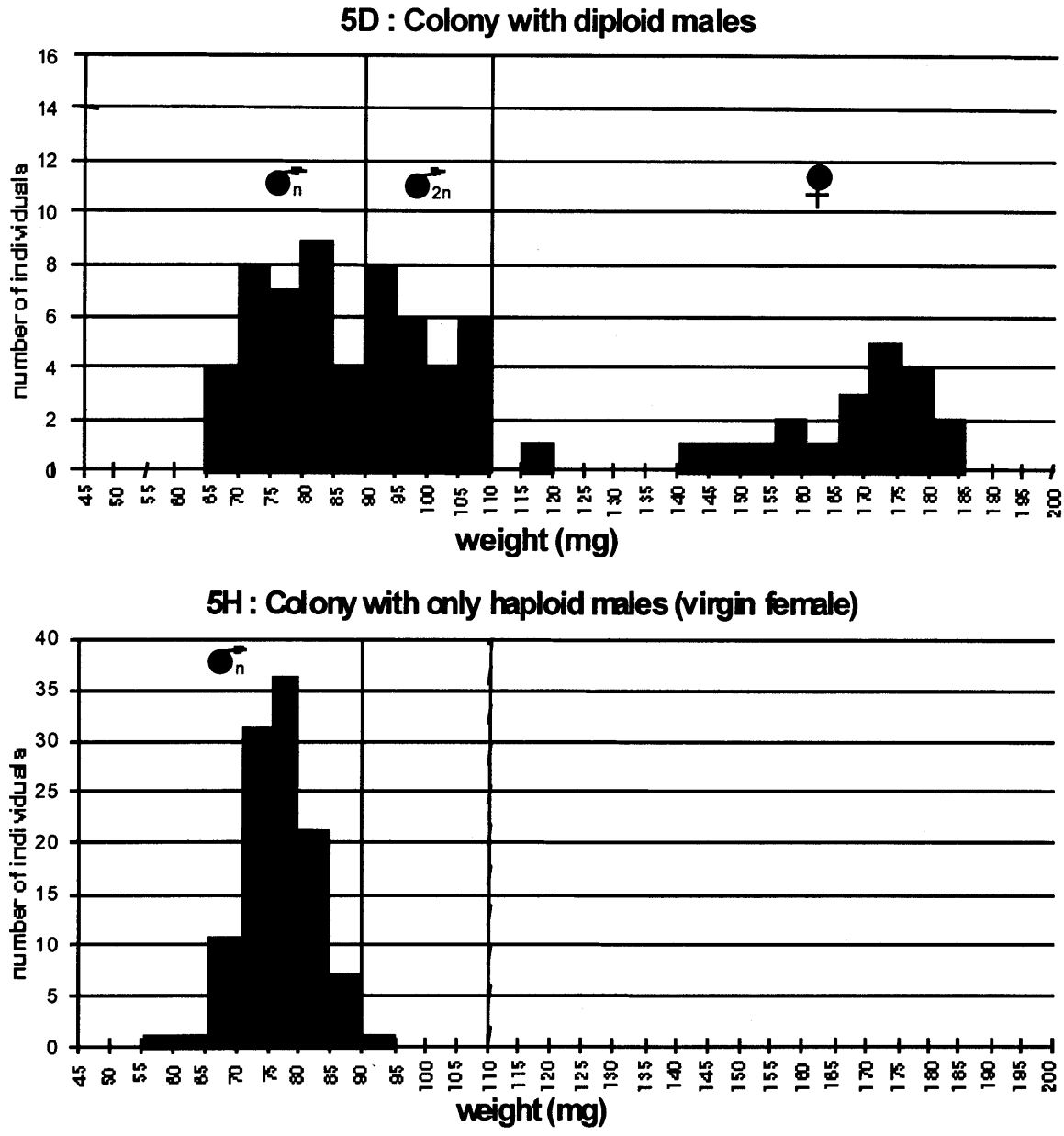

Figure 4. Distribution of prepupae belonging to colonies with or without diploid males as a function of cocoon weight.

Sex Determination. It is generally very difficult to succeed in mating and to obtain a sufficient number of colonies descended from the same grandmother (P) to test the expected proportions (50%-50% or 25%-75%). Many brother-sister crosses didn't succeed and produced progenies with only haploid males from unfertilized eggs. Furthermore, we were confronted with high diapause and mortality rates.

Our data does not permit us to arrive at a conclusion concerning sex determination models. The two experiments show that two-locus CSD is more likely with regard to the proportion of colonies containing diploid males, whereas the diploid male proportion is

consistent with the single-locus model. In the second experiment, for example, two colonies among eight progenies contained diploid males. It's completely consistent with the two-locus model (25%-75%). On the other hand, if diploid males are as viable as females, the expected proportions are 50%-50% under the single-locus model and 25%-75% under the two-locus model. In this experiment, there are approximately as many diploid males as females (53%-47%). Furthermore, diploid males are generally known to be less viable. So, single-locus CSD seems more likely from this point of view.

Possible Impact of Diploid Males on Population Dynamics. It is well known that diploid males affect the population biology of Hymenoptera (Cook and Crozier 1995). They are usually sterile or their sperm are mostly diploid and their progeny are triploid and not viable (except in the sawfly *Athalia rosae* - Naito and Suzuki 1991). Furthermore, they reduce population growth because they develop from eggs that are "normally" female.

With regard to *D. pini,* it is assumed that diploid males could interfere in diapause rates (Géri et al. 1995). On the other hand, it is possible that the very small size of *D. pini* populations between outbreaks entails a high level of consanguinity which could entail a considerable production of diploid males. Consequently, diploid males would reduce population growth and maintain a low population level. In this scheme, diploid male production would be stopped only when new alleles enter into the population (by migration or prolonged diapause emergences) thereby starting outbreaks.

Conclusion

It is concluded that diploid males occur in *D. pini* and we can suppose that these males play a prominent part in population dynamics. With the use of the sex pheromone recently synthesized (Bergström et al. 1995), it is now possible to sample males in the field by pheromone trapping in order to estimate their frequency in the natural population. However, testing our hypothesis requires more advances in the identification of diploid male adults and in the study of their biology (especially their mating ability, sterility, ploidy level of sperm, viability and larval development).

It is also necessary to settle the problem of sex determination, because single-locus CSD is more consistent with a high frequency of diploid males in the natural population. So, it is essential to confirm the karyotype evolution by centric fission because chromosome number doubling by polyploidization assumes the existence of (a minimum of) two sex loci.

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Noxious Effect of Scots Pine Foliage on *Diprion pini* L. (Hym. Diprionidae) and Other Defoliators: Specificity and Prospects for Use in Forest Management

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ABSTRACT Feeding bioassays were conducted with the foliage of Scots pine clones, previously identified as favorable or unfavorable to the sawfly *Diprion pini*, in order to observe their effect on different populations of *D. pini* and on two lepidoptera defoliators, *Thaumetopoea pityocampa*, an important and dangerous pest, and *Graellsia isabellae*, a scarce and protected moth. All populations of *D. pini* consumed less foliage on the unfavorable clones; variation in consumption was related to the age of the larvae and to the season of the experiments. Similarly, *T. pityocampa* mortality increased and its larval development slowed down. Biological performances of *G. isabellae* were disturbed, too, and in this case, consumption of favorable clones allowed this species to obtain better survival and development rates than in nature. These results are discussed with regard to Scots pine breeding.

THE LARVAE OF the pine sawfly, *Diprion pini*, cause dramatic defoliation on Scots pine, its main host in Europe. In France, infestations occur especially in plains, where the insect is bivoltine (Géri 1988). The effect of the foliage on *D. pini* mortality, development rate, weight, fecundity and diapause has already been investigated using bioassays that involved starvation (Géri et al. 1988a), age (Géri et al. 1985, 1988a,b) and quality of the foliage (Géri et al. 1990). Auger et al. (1990, 1991, 1994) showed the existence of an interclonal intraspecific variability. Scots pine clones which are very unfavorable to the development of this insect were discovered in breeding populations of Polish origin. In order to determine the chemical compounds of the needles which could be associated with these noxious properties, analyses were conducted on polyphenols. The phenolic content of the foliage of Scots pine clones unfavorable to the larval development and survival of *D. pini* was characterized by the presence of two flavonoïds: taxifolin and one of its glucosides. These compounds were absent from the majority of the French clones and from some Polish clones favorable to the insect's performance (Auger et al. 1992, 1994). Furthermore, an induced reaction was shown when Scots pine trees were defoliated. Defoliation resulted in a decrease in the amount of needle taxifolin and an increase of its glucoside. However, this response is not limited to defoliation by *D. pini* larvae; Bastien et al. (1995) and Auger et al. (1994, and unpublished results) observed the same results after artificial defoliation with scissors. The reaction of the trees seemed to be localized near the wound. Nevertheless, it is currently difficult to evaluate the impact and mode of action of these phenolic compounds and the exact part they could play in Scots pine resistance mechanisms to insect pests. Because of the prospective use of

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resistant Scots pine in forest management, it was important to know the potential noxiousness of those clones for different populations and species of defoliators.

This article discusses experiments conducted to investigate clone foliage toxicity effects on different populations of *D. pini* and on two other Lepidopteran Scots pine defoliators: *Thaumetopoea pityocampa* Schiff. (Lep. Thaumetopoeidae) and *Graellsia isabellae* Graells (Lep. Attacidae). *Thaumetopoea pityocampa* is an economically important pest in Mediterranean forests and dermal irritations caused by larvae hair are harmful to humans. *Graellsia isabellae* is a scarce and protected beautiful moth located in the South Alps. Scots pine clones were used in feeding bioassays with *D. pini* larvae and associated with the phenolic content of the needles. In order to observe the specificity of foliage noxiousness, the effect of the foliage was comparatively observed on *D. pini* populations from different origins. Clones were then tested with caterpillars of *T. pityocampa* and *G. isabellae*.

Materials and Methods

Plant Material. Different clones of Scots pine of French (Alsace) and Polish (Mazurie) origin, used as breeding populations in an INRA breeding programme conducted at Orléans Station, were used in the following experiments. Each clone, identified by a code number, is represented by several grafted copies present at Orléans.

Feeding Bioassays With *D. pini*. Different bioassays, including several experiments and repetitions, were conducted from 1990 to 1993 with first instar larvae (which were more sensitive to feeding conditions) from a permanent laboratory strain reared from generation to generation (Goussard and Géri 1988) and from field populations near Orléans. Batches of 30 larvae were formed. The larvae were placed in transparent polystyrene boxes, which were closed with lids that allowed aeration, then placed in a growth chamber (15.30 / 8.30 h photoperiod, 16°C temperature). They were fed every 3 days with shoots taken from clones of the INRA nursery. The foliage was renewed by placing new shoots on the preceding ones, which were removed when the larvae had all moved to the fresh shoot. Larvae were fed for 12 days, and larval mortality rates were determined at the end of the test. Up until now, 37 clones were tested for their edibility for young *D. pini* larvae. For some clones, tests were completed by feeding bioassays using older larvae, in order to measure other biological characteristics, such as development rates, pupal weight, diapause rate and fecundity (Auger et al. 1990). We defined two groups of clones: one group of unfavorable clones and one group of favorable clones.

In 1994 and 1995, populations of *D. pini* larvae were collected in different forests and fed with different clones using the same method described above (two batches of 30 larvae per clone). They were collected at the first instar just after hatching or at the end of the first instar in the Fontainebleau forest (in the Parisian Basin), in the Alps and in a population of Finish origin, and immediately used in feeding tests. Feeding tests were conducted with the rearing strain at the same time for comparison.

Feeding Bioassays With *T. pityocampa*. *T. pityocampa* caterpillars live in colonies. They cause considerable damage, especially on black pine, but also attack Scots pine. From the 4th instar, they are highly irritating to humans and animals. This pest is present in southern France; the Orléans region represents the northern limit of its distribution. Two

experiments were conducted during the fall of 1992 and 1994. In 1992, colonies of first instar larvae (each of them corresponding to one egg laying) were collected in forests near Orléans after eggs were counted. Then, they were placed in the field on the branches of 10 clones: four clones (356, 632, 721 and 864) unfavorable to the survival and development of *D. pini* and six clones (129, 20, 16, 15, 14 and 13) that were favorable. Two colonies (one per tree) were placed on two trees per clone. In 1994, we used the same method with four clones: two favorable (16, 129) and two unfavorable (147, 136). Three colonies were placed on three trees per clone (one colony per tree) under natural conditions. In order to avoid climatic risks and itches, colonies were collected when larvae reached the fourth instar. Mortality and the development rate of the caterpillars were estimated by counting the survivors and observing the larval instars affected.

Feeding Bioassays With *G. isabellae*. This beautiful moth is known in Spain and in France where the subspecies *galliegloria* Oberthür, located in some valleys of the South Alps, is protected. Scots pine is its main host. Feeding bioassays were performed with a population reared at INRA since 1990, from eggs given by the OPIE (Office of Entomological Information) without catching insects in the wild. Caterpillars were fed cut shoots in transparent boxes as previously described from the time of hatch to cocoon spinning and kept in an outside shelter. Feeding bioassays were performed in the spring of 1992, 1993, 1994 and 1995. In 1992 and 1993, caterpillars were fed with five clones and for each clone, two batches of five caterpillars were formed: three unfavorable clones (356, 588 and 721) and two favorable (129 and 14) in 1992, and two unfavorable clones (627 and 721) and three favorable (129, 614 and 875) in 1993. In 1994, eight clones were used (721, 646, 588, 356 and 649 unfavorable and 129, 14 and 875 favorable). For each clone, four batches of five caterpillars were formed except for clones 875, 721 and 356 (two batches of five caterpillars). In 1995, three clones were used (147 unfavorable, and 129 and 20 favorable) with twelve batches of eight caterpillars for each clone. The biological characteristics of *G. isabellae* taken into account were: the percent larval survival, the sex ratio (% male cocoons), the length of larval development and the weight of the male and female chrysalis.

Biochemical Methods. In order to analyze the phenolic content, needle samples were collected at random in the trees before feeding bioassays, frozen immediately after collection in liquid nitrogen and then freeze-dried and ground to a powder before storage in dry conditions under a vacuum. The phenolic compounds were analyzed by HPLC and the presence or absence of the two compounds, identified as taxifolin and taxifolin glucoside, was observed for the whole clone employed in feeding bioassays. Extraction, elution programme and identification modalities are described by Auger et al. (1994).

Results

Experiments On *D. pini*. The percentages of young larvae surviving after 12 days of feeding on the different clones from 1988 to 1993 and the presence or the absence of taxifolin in the needles (when the phenol content of the foliage was analyzed) are shown on Figure 1. We can observe an important interclonal variability and higher mortality with clones containing taxifolin and its glucoside.

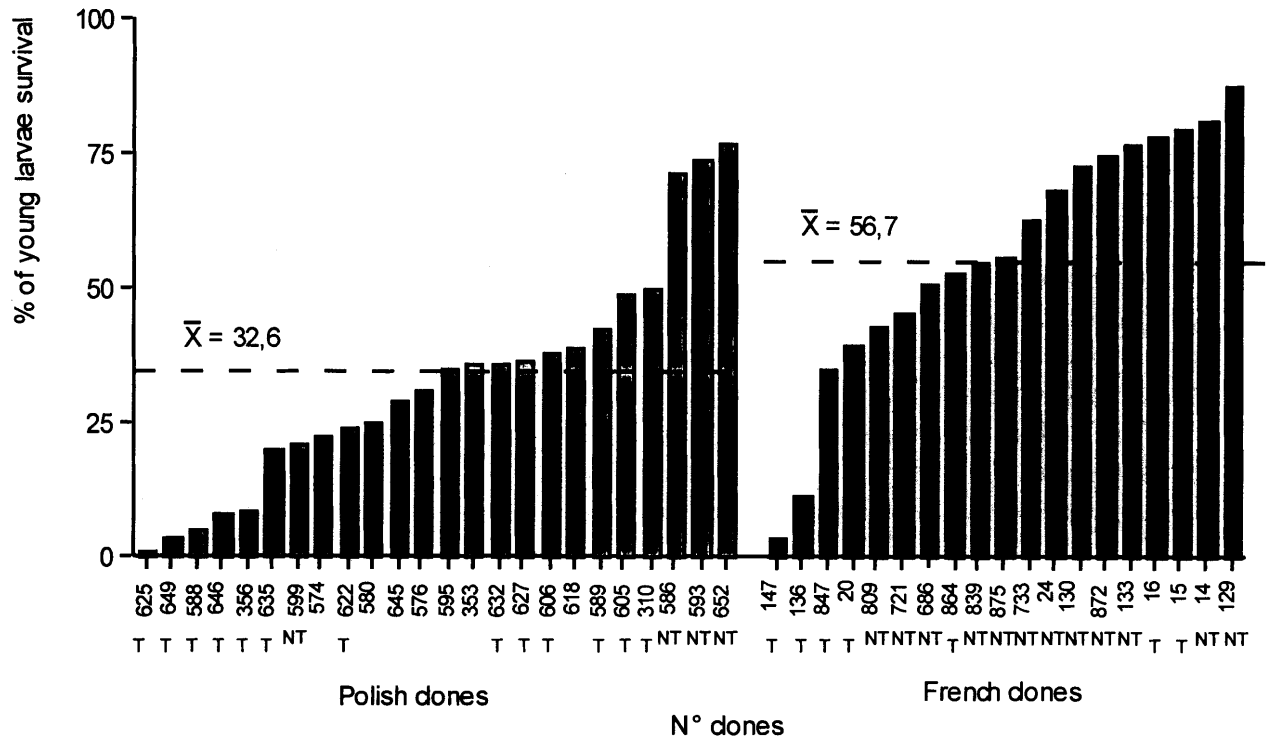

Figure 1. Mean survival (%) of *D. pini* larvae when young larvae fed in the laboratory on several Scots pine clones. Experiments were conducted from 1988 to 1993. For clones whose phenol content was analyzed: T = with taxifolin and taxifolin glucoside; NT = without the compounds.

Table 1 shows the results of feeding bioassays conducted with different populations of *D. pini* in 1994 and 1995. Mortality is always higher for larvae fed with unfavorable clones, whatever the origin of the population or the date of the test. When surviving larvae existed on unfavorable clones, we noted a decline in larval development and a decrease of both diapause rate and female fecundity. However, if the edibility of clones which were previously classified as very favorable (129) or very unfavorable (646, 588) is quite stable, differences existed for some clones with regard to the age of larvae, the season when tests were conducted, and the origins of *D. pini* populations. Thus, clone 20 caused high mortality of larvae from the rearing strain or Finland, but not of larvae from the Alps or the Fontainebleau Forest; clones 147 and 136 also showed variable mortality depending on populations. Whatever the population, foliage was unfavorable to larvae in November and mortality was important even with favorable clones (129). On the other hand, mortality was higher when the test was performed with L1 than with older larvae (L2). The effect of the intermediate clones on different populations needs to be confirmed.

Table 1. Feeding bioassays conducted in 1994 and 1995 with different populations of *D. pini*. Mean survival of larvae fed with different Scots pine clones. G: clones favorable to *D. pini* survival B: clones unfavorable to *D. pini* survival. T: clones with taxifolin and taxifolin glucoside. NT: clones without the compounds

Date of bioassays	Origin of *D. pini* population	Larval instar	N° clones							
			733 G NT	129 G NT	872 G NT	20 G NT	16 B T	147 B T	136 B T	646 B T
06/07/94	Rearing strain	L2	71,7							
13/09/94	Rearing strain	L1	50	66,3	53,3				0	
01/03/95	Rearing strain	L1		53,3		2,5		5		
15/11/94	Alps	L1		20	50		0	0		0
21/11/94	Alps	L2		76,7			6,7		0	0
21/04/95	Alps	L1		89,4		82,7		56,7		
03/03/95	Fontainebleau	L1		76,7				56,7		
10/03/95	Fontainebleau	L1		75		82,5		35		
18/10/94	Finland	L2		85	58,3				73,3	15
21/04/95	Finland	L1		71,7		30		26,7		

Experiments On Other Scots Pine Defoliators. The results of feeding bioassays on *T. pityocampa* (Pine processionary) are presented in Figure 2 for the experiments conducted in 1994. Important significant variations in caterpillar mortality and length of development were shown among the different clones. Foliage unfavorable to survival and development of *D. pini* larvae caused high mortality and slower larval development. The same results were observed in 1992; however, clone 864 (unfavorable to *D. pini*) caused only a drastic decrease of the larval development rate, and clone 14 (favorable to *D. pini*) caused high mortality. Therefore, we observed variability between different clones, but mortality was always higher with unfavorable clones than with favorable clones and linked with the presence of taxifolin.

The results of feeding bioassays on *G. isabellae* caterpillars were very consistent from year to year. They are presented in Figure 3 for the year 1994. During the four years, the effect of the consumption of clones was highly significant for all biological characteristics studied. The clones that were unfavorable to *D. pini* were unfavorable to *G. isabellae* caterpillars. They caused higher mortality (but not as high as that for *D. pini*), slower larval development and smaller pupal weight, whatever the year of the experiments. The worst performances (survival and development) were always observed with very unfavorable clones and, conversely, clones favorable to *D. pini* produced lower mortality, faster development and heavier *G. isabellae*. Very heavy female chrysalises, superior in weight to those observed in natural populations, were obtained when insects were fed clone 129, which was also very favorable for *D. pini*. As in the case of the previous species, performances of clones with *G. isabellae* were linked with the presence of taxifolin in the needles.

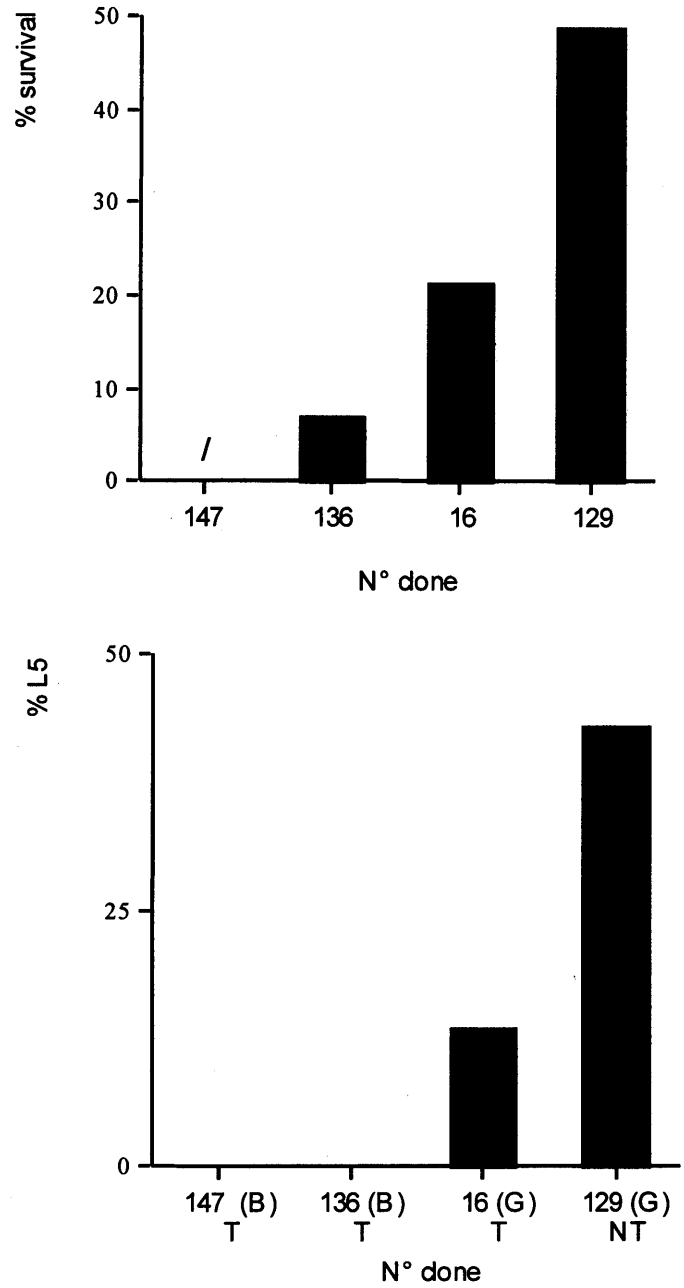

Figure 2. Mean larval survival and mean duration of development for caterpillars of *T. pityocampa* reared on different Scots pine clones in natural conditions. Experiments were conducted in 1994. B: clones unfavorable to *D. pini* survival. G: clones favorable to *D. pini* survival. T: clones with taxifolin and taxifolin glucoside. NT: clones without the components.

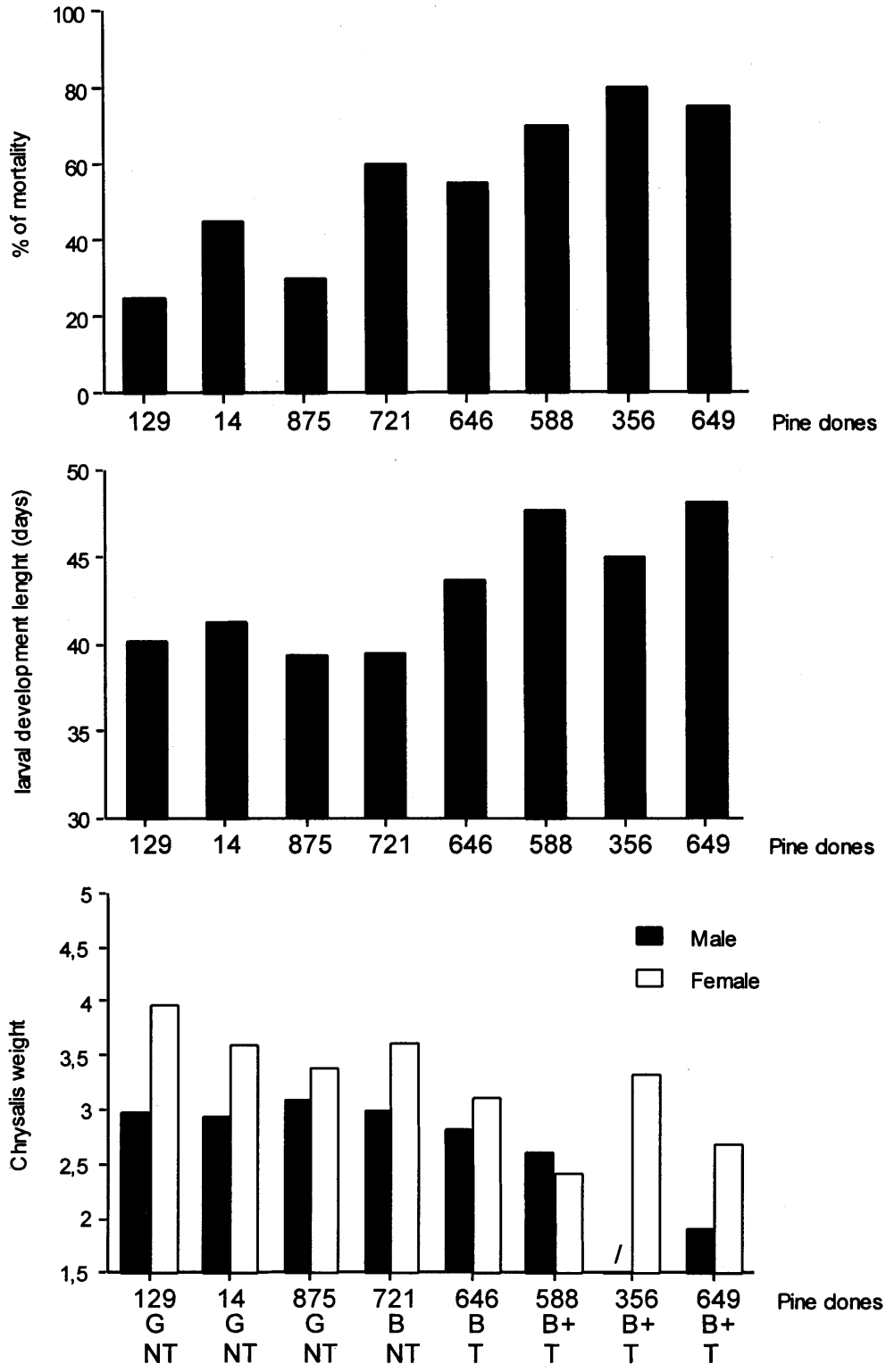

Figure 3. Performances of *Graellsia isabellae* fed with different Scots pine clones (experiments conducted in 1994). G: clones favorable to *D. pini* suvival. B: clones unvaforable to *D. pini* survival. B+: clones very unfavorable to *D. pini* survival. T: clones with taxifolin and taxifolin glucoside. NT: clones without the compounds.

Discussion and Conclusion

The results of these experiments on feeding show that the noxiousness of Scots pine clones is rather non specific and stable, not only for various populations of *D. pini*, but also for distant species of Scots pine defoliators. However, this needs to be confirmed with other pests.

The interclonal variability is notable for four different populations of *D. pini*, but needs to be verified by feeding tests with other populations. Some differences exist between populations, allowing one to assume that individual variations in the aptitude of insects to eat different foliage and perhaps genetic possibilities to overcome needle noxiousness exist. However, those differences need more precise investigations that consider others factors, such as insect age and seasonal variations in foliage quality. Nevertheless, clones previously noted for very favorable or very unfavorable characteristics presented homogenous results. We have to note that larvae of the rearing strain, usually fed with favorable foliage, showed higher mortality rates than larvae of field populations.

The results of feeding tests with *T. pityocampa* must be treated with caution because of the small number of experiments. Only first larval instars were observed and the tests were conducted under natural conditions, which confound the foliage effect. However, even if tests conducted at the end of insect development have to be made to prove the effect of clones on biological characteristics, such as pupal weight or fecundity, our results are in accordance with clone aptitudes observed with *D. pini*. Big differences between clones were shown for caterpillar mortality and development length. For *G. isabellae*, the results were repeated each year, and, as with *T. pityocampa*, the results clearly indicate the effects of Scots pine clone consumption on survival and development: their biological performances can be disturbed by consumption of clones which are known to be unfavorable to *D. pini*. The feeding effects of unfavorable clones were less important for *G. isabellae* than for the two insect pests used in the tests, but we have to be careful with the use of clone progeny obtained in a breeding program. They have a general effect on total fauna and they don't have to be introduced in the living area of this protected species. Moreover, some clones lead to really good insect survival and development, especially for chrysalis weight directly linked with fecundity, that were better than we can observe in nature. Those favorable clones can be used to rear scarce and protected species. However, we must ask ourselves if those clones wouldn't also be very favorable to the development of different insect pests. If breeding programs don't take this into account, trees selected for good growth criteria could also be very favorable for the breeding of efficient pests.

The species studied belong to very different families and these results are really interesting with regard to prospective use in forest management. In fact, if breeders want to obtain resistant trees, it is important to appreciate their effect on different species of defoliators. But environmental consequences on non-pest species and risks to select resistant insects need to be considered. It's comforting to note that the least affected species is the protected one.

Nevertheless, breeding strategy for Scots pine resistance is still to be achieved, and there is a need to experiment with others pests and to consider the long-term adaptations of insects to unfavorable trees.

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Sensitivity of the More Important Forest Defoliating Insects in Poland to Insecticides

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ABSTRACT The susceptibility of the most important forest defoliating insects, such as nun moth, *Lymantria monacha* L., pine moth, *Dendrolimus pini* L., pine beauty moth, *Panolis flammea* Schiff. and conifer sawflies, Diprionidae, to approved and experimental insecticides (pyrethroids, arylpropylether, acylureas, diacylhydrazine) and biopreparations (based on a bacterium, *Bacillus thuringiensis* Berliner) was evaluated under laboratory conditions. Two methods were used: topical application and exposure of insects to treated pine needles.

Results indicated that although there is some evidence of resistance to frequently used insecticides (mainly to pyrethroids) in some insect populations, the above species can be controlled effectively with pyrethroid insecticides. The studies also showed that the insect populations we studied are fully sensitive to acylurea and diacylhydrazine insecticides and biological preparations based on *B. thuringiensis*. However, the sensitivity of target pest species to chemical and biological insecticides should be evaluated regularly.

THE MOST SERIOUS forest pests in some European countries, especially in Poland, include the nun moth, *Lymantria monacha* L., pine moth, *Dendrolimus pini* L., pine beauty moth, *Panolis flammea* Schiff., and conifer sawflies, family Diprionidae. Their periodic outbreaks often affect vast areas of Scots pine, *Pinus sylvestris* L., and Norway spruce, *Picea abies* Link., and can cause severe damage. To protect forests effectively against the above mentioned pests, chemical and biological insecticides are frequently used. For background information, a short history of the use of insecticides in Poland for control of forest defoliation is provided. Between 1950 and 1980, chlorinated hydrocarbons (DDT, lindane, methoxychlor) were most frequently used to control forest defoliators; in addition, organophosphates and carbamates were also used between 1974 and 1980. However, since 1980, pyrethroids such as deltamethrin and alphamethrin have been used most frequently against defoliating insects.

Although biopreparations of *Bacillus thuringiensis* (Foray, Dipel, Ecotech Pro) and acylureas (diflubenzuron, teflubenzuron, triflumuron) have been available since 1990, these products were rarely applied or used on smaller areas. Therefore, it can be assumed that among the insecticides used since 1980, the selection pressure of the pyrethroids to forest pest defoliators has been greater. The purpose of these studies was to evaluate the sensitivity of our most important forest defoliating insects to a variety of operational and experimental insecticides under laboratory conditions.

Pages 100-107 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

Materials and Methods

The following species of insects were used in laboratory bioassays:

- nun moth (*Lymantria monacha* L.) - second and third instars
- pine moth (*Dendrolimus pini* L.) - first through fourth instars
- conifer sawflies (Diprionidae) (mixed population of different species of Diprionidae, mainly *Diprion pini* L. and *Gilpinia frutetorum* F.) - second instars
- pine beauty moth (*Panolis flammea* Schiff.) - second and third instars and their parasites: *Rictichneumon pachymerus* Katz. and *Barichneumon bilunulatus* Grav.

Larvae of the above mentioned insect species were collected in Scots pine stands from different forest districts in Poland between 1990 and 1995.

The insecticides used were:

Pyrethroids

- alphamethrin as 100 g/l EC (Fastac 100 EC, Shell)
- bifenthrin as 100 g/l EC (Talstar 100 EC, FMC)
- betacyfluthrin as 25 g/l EC (Bulldock 025 EC, Bayer)
- deltamethrin as 25 g/l EC (Decis 2,5 EC, Roussel - Uclaf)
- esfenvalerate as 50 g/l EC (Sumi - Alpha 050 EC, Sumitomo)
- lambdacyhalothrin as 25 g/l EC (Karate 025 EC, Zeneca)
- zetacypermethrin as 100 g/l EC (Fury 100 EC, FMC)

Arylpropylether

- etofenprox as 100 g/l SC (Trebon 10 SC, Mitsui Toatsu)

Acylureas

- diflubenzuron as 480 g/l SC (Dimilin 480 SC, Solvay Duphar)
- flufenoxuron as 50 g/l EC (Cascade 5 EC, Shell)
- novaluron as 100 g/l EC (GR 572 EC, Agrimont)
- teflubenzuron as 150 g/l SC (Nomolt 15 SC, Shell)
- triflumuron as 480 g/l SC (Alsystin 480 SC, Bayer)

Diacylhydrazine

- tebufenozide as 240 g/l EC (Mimic 24 EC, Rohm and Haas)

Biopreparations of *Bacillus thuringiensis*

- Foray 48B (B.t. kurstaki, potency min. 11.000 i.u./mg, Novo Nordisk)
- Ecotech Pro 07.5 OF (strain EG 2348, transconjugant *Bt* kurstaki x *Bt* aizawai, potency 24,000 i.u./mg, Ecogen)

Two methods were used to test the sensitivity of insects to contact insecticides: direct topical application to larvae, and exposure of insects to treated pine needles. For stomach poisons such as *Bt* and acylureas, insects were exposed to treated pine needles.

Topical Method. Insecticides were dissolved in acetone to produce six to eight different concentrations (doses). Larvae were individually treated using the Arnold microapplicator. A 1-µl drop of insecticide solution was applied to the dorsal surface of larvae. Percent mortalities were calculated 48 or 76 hrs after application of insecticides. Controls were treated with acetone. Mortality data were corrected for mortalities in the control insects according to Abbott's formula and subjected to probit analysis (Finney 1952).

Exposure of Insects to Treated Pine Needles. Aqueous suspensions of each insecticide were prepared at seven different concentrations. Bouquets of Scots pine needles, about 15 cm long, were dipped for five seconds in each concentration and allowed to dry. The test larvae were placed on the treated foliage, whereupon the bouquets were placed in water under glass tubes covered with cloth. Two replications of 15 larvae each were used for each concentration. The same procedure was used for controls that were treated with water only. In the case of the pine beauty moth, the caterpillars were exposed to treated filter paper with deltamethrin only. Percent mortalities were calculated after 48 hours (pyrethroids and etofenprox), 4 to 6 days (*B. thuringiensis* biopreparations) or 10 to 12 days after treatments (acylureas and tebufenozide).

Calculations of percent mortality included both dead and severely affected individuals. Tests were conducted at a temperature of 20-25°C for all treatments.

Results and Discussion

Susceptibility of Insects to Pyrethroids and Arylpropylether (Etofenprox). Topical Application Method. The topical application method allowed us to produce regression lines that demonstrated the relationship between doses and mortalities. Data on the susceptibility of second to third instar nun moth larvae to pyrethroids and etofenprox were presented earlier (Malinowski 1996). Those data showed that nun moth caterpillars exhibited similar response to most pyrethroids, resulting in regression lines that were close together. Nun moth larvae responded similarly to etofenprox; however, higher doses were required to cause comparable levels of mortality (Malinowski 1996).

The sensitivity of third to fourth instar pine moth larvae to pyrethroids and etofenprox is illustrated in Figure 1. The shape of the regression lines with small slopes for pyrethroids is similar to that observed in nun moth larvae. LD_{50} values ranged from 0.0017 μg/individual (deltamethrin) to 0.004 μg/individual (bifenthrin), resulting in two-fold differences. The regression line for etofenprox is also placed in the scale of higher doses and its slope is similar to that of pyrethroids.

A similar situation was observed in the case of conifer sawflies, Diprionidae, treated with pyrethroids and etofenprox (Fig. 2). The slope of the regression lines for pyrethroids and etofenprox is similar to other forest defoliating insect species discussed previously. However, LD_{50} values between 0.00014 μg/individual (deltamethrin) and 0.00031 μg/individual (bifenthrin) were lower than those obtained from studies on pine moth larvae (Fig. 1). Diprionid larvae were less sensitive to etofenprox than to pyrethroids, which is similar to the results with other species.

These studies showed that in all cases, the slope of the regression lines for pyrethroids is similar. Some differences in LD_{50} values were observed among species; however, for a given species, the LD_{50} values between pyrethroids differed only about two-fold. These studies also showed that in all cases, the slope of the regression lines for etofenprox is similar to those for pyrethroids; however, higher doses were required to produce a level of mortality similar to the pyrethroids. The shape of the regression lines with small slopes for both pyrethroids and etofenprox indicates that a degree of heterogeneity exists among individuals of all species studied. These populations of insects are composed of a mixture of resistant,

sensitive and hybrid individuals, and the quantitative relationships between them determine the shape of the regression lines. This is caused by selection pressure of insecticides currently being applied, such as pyrethroids, and probably by other products that were used previously, for example DDT. It has been suggested that cross resistance between DDT and pyrethroids exists (Malinowski, 1988).

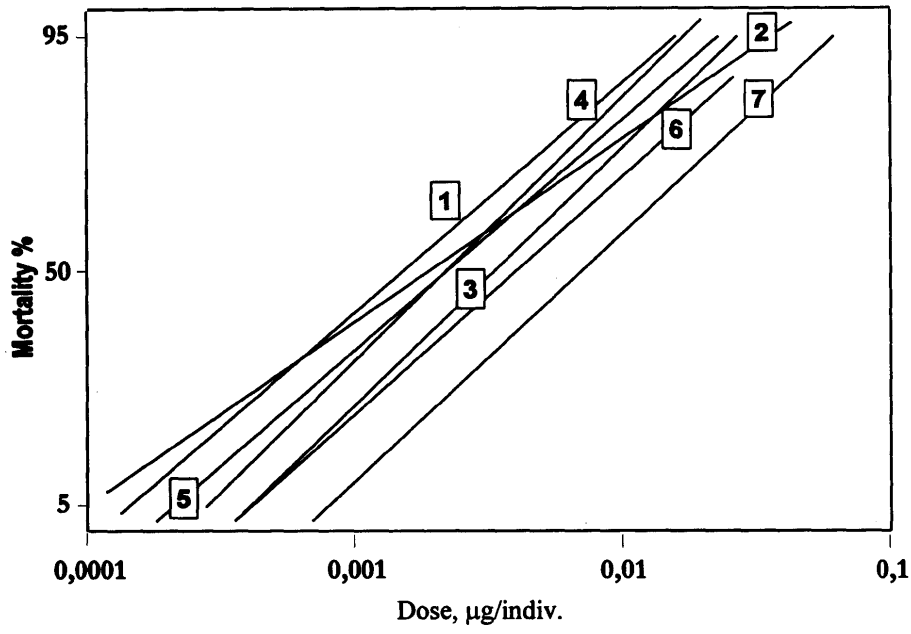

Figure 1. Regression lines obtained from studies on pine moth caterpillars L3/L4 instars treated topically with insecticides: 1-deltamethrin, 2-esfenvalerate, 3-zetacypermethrin, 4-lambdacyhalothrin, 5-alphamethrin, 6-bifenthrin, 7-etofenprox. Topical application method, assessment after 48 hrs. 1,2,5-studies on populations from Bialkow Forest District (1991, 1992), 3,4-studies on populations from Krosno Forest District (1993) and 6,7-studies on population from Ostrow Mazowiecka Forest District (1993).

Exposure of Insects to Treated Pine Needles. Although the insect populations tested are characterized by their heterogeneity in response to pyrethroids and etofenprox, they can be controlled effectively with these insecticides. Our studies conducted with the dipping technique showed that nun moth larvae that were exposed to a range of concentrations of pyrethroids and etofenprox suffered 100% mortality at three (0.01, 0.001, 0.0001 g/l) and two (0.01, 0.001 g/l) of the highest concentrations, respectively (Malinowski 1996). Similar results were obtained from studies on larvae of the pine moth and conifer sawflies. These results confirmed the data obtained by the topical application method which showed that pyrethroids were more effective than etofenprox against the larvae tested.

The comparative evaluation of the sensitivity of pine beauty moth larvae and their parasites *Rictichneumon pachymerus* Katz. and *Barichneumon bilunulatus* Grav. to deltamethrin, by exposing them to treated filter paper, showed differences between parasites and their host (Glowacka and Malinowski 1991). Parasites were less sensitive to deltamethrin

than as their host. Both parasites suffered 100% mortality only at the highest concentration (1 g/l) of deltamethrin. In the case of pine beauty moth larvae, 100% mortality was obtained at a 1000-fold lower concentration of deltamethrin. Results of this study also indicated that pine beauty moth caterpillars originating from different localities differed in their response to deltamethrin. It may be assumed that the lower sensitivity of some pine beauty moth caterpillars to deltamethrin can be attributed to the intensive use of pyrethroids in the past.

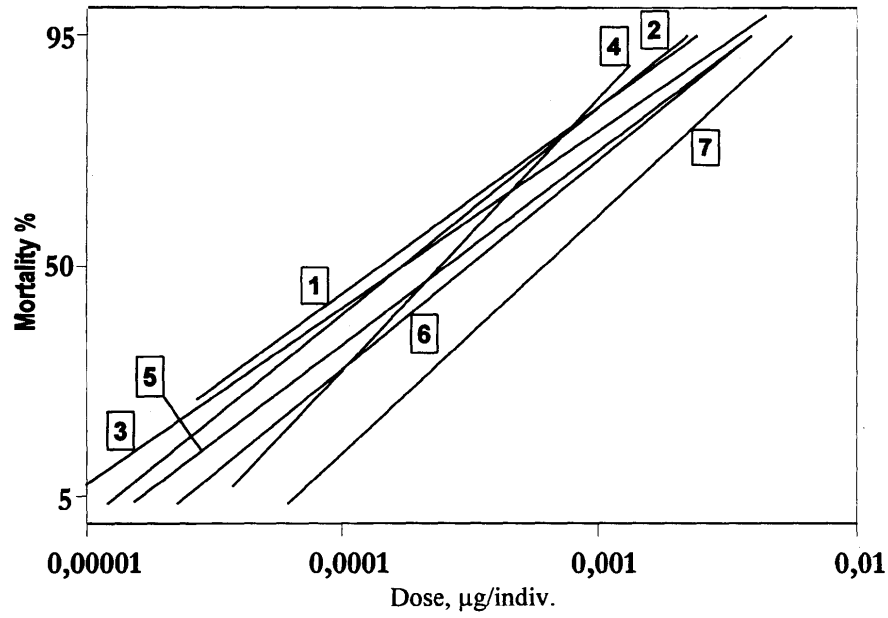

Figure 2. Regression lines obtained from studies on *Diprionidae* L2 instar larvae treated with insecticides: 1-deltamethrin, 2-zetacypermethrin, 3-alphamethrin, 4-lambdacyhalothrin, 5-esfenvalerate, 6-bifenthrin, 7-etofenprox. Topical application method, assessment after 48 hrs. 1,3,5-studies on populations from Bialkow Forest District (1991), 2,4,6,7-studies on populations from Podrodzie Forest District (1992, 1993).

Susceptibility of Insects to Acylureas. All larvae of species tested were susceptible to acylurea compounds such as diflubenzuron, flufenoxuron, novaluron, teflubenzuron and triflumuron (Malinowski 1995a). Nun moth larvae were equally susceptible to flufenoxuron, novaluron, teflubenzuron and diflubenzuron (Malinowski 1996). These compounds gave 90% larval mortality at a concentration of 0.0001 g/l, while 90% mortality with triflumuron occurred at a higher concentration (0.001 g/l). The response of pine moth and sawfly larvae (*Diprionidae*) (Table 1) was similar to that of nun moth larvae with one exception: diflubenzuron was effective at a higher concentration of 0.001 g/l. This group of insecticides was introduced more recently for control of forest insects and has been used on a small scale. Therefore, their selection pressure was too weak to produce resistant individuals in our test populations of insects.

Table 1. Toxicity of acylurea insecticides for L_2 instars of conifer sawfly (Diprionidae) larvae exposed to treated pine needles

Insecticide	LC_{90}*
flufenoxuron	0.0001
novaluron	0.0001
teflubenzuron	0.0001
diflubenzuron	0.001
triflumuron	0.001

*Concentration (g/l) giving minimum 90% population mortality after 10-12 days

Susceptibility of Insects to Tebufenozide. Tebufenozide (RH-5992) is a novel synthetic nonsteroidal ecdysteroid agonist, which represents a new class of insect growth regulators. It induces a premature and lethal larval molt by direct stimulation of the ecdysteroid receptors, especially in *Lepidoptera*. In this respect, it mimics the activity of the natural ecdysteroids (Heller et al. 1992, Smagghe and Degheele 1994, Slama 1995).

The sensitivity of *D. pini*, *B. piniarius* and *L. monacha* larvae to tebufenozide was tested in Poland under laboratory and field conditions (Malinowski and Glowacka 1996). Results of laboratory experiments using both methods--topical application to larvae and exposure of insects to treated pine needles--are given in Table 2. The data showed significant activity of tebufenozide against larvae of the above mentioned species of forest *Lepidoptera* at low concentrations.

Table 2. Toxicity of tebufenozide to larvae of some forest pest Lepidoptera

Pest	Method	Dose causing minimum 90% mortality
Dendrolimus pini		
larvae of second instar	exposure of insects to treated pine needles	0.001-0.0001%
larvae of first instar	topical application	$LD_{50} < 1\mu g$/larva
Bupalus piniarius		
larvae of second instar	exposure of insects to treated pine needles,	0.0001%
	topical application	$LD_{50} < 1\mu g$/larva
Lymantria monacha		
larvae of second instar	exposure of insects to treated pine needles	0.0001%

More detailed studies conducted in our laboratory (Pszczólkowski, personal communication) on the efficacy of tebufenozide against different larval stages of the pine

moth, using the same methods, gave the following results: the LD_{50} for L_5-L_6 instars was approximately 0.001 g/L (dipping technique, 10 days after treatment) and the LD_{50} for L_7 instars was between 0.004 - 0.04 µg/larva (topical method, 10 days after treatment).

Susceptibility of Insects to Biopreparations of *Bacillus thuringiensis*. Biopreparations of *B. thuringiensis*, which are typical stomach poisons that must be ingested by larvae, have been evaluated by Malinowski (1995b). Results of tests conducted on Foray 48 B against both nun moth and pine moth larvae (Fig. 3) suggest that both species are susceptible to this product.

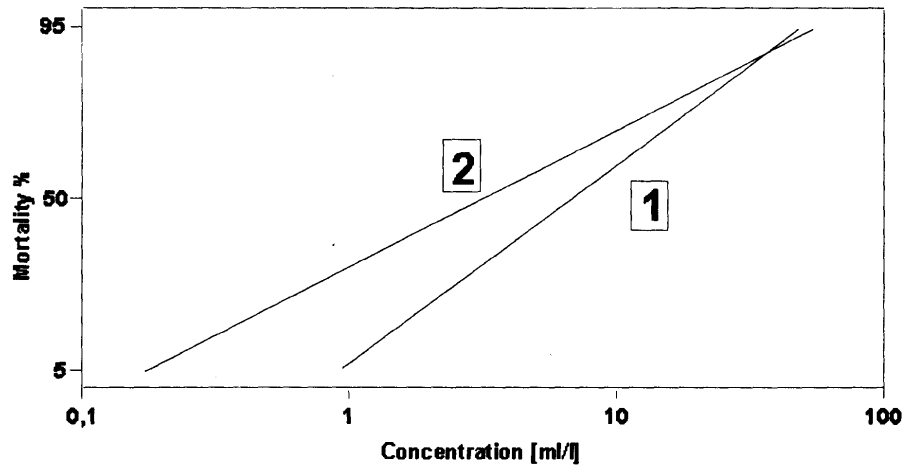

Figure 3. Regression lines obtained from studies on Lepidoptera caterpillars exposed to pine needles treated with Foray 48 B. (1) L2/L3 instars of nun moth caterpillars (assessment after 6 days), (2) L1/L2 instars of pine moth caterpillars (assessment after 4 days).

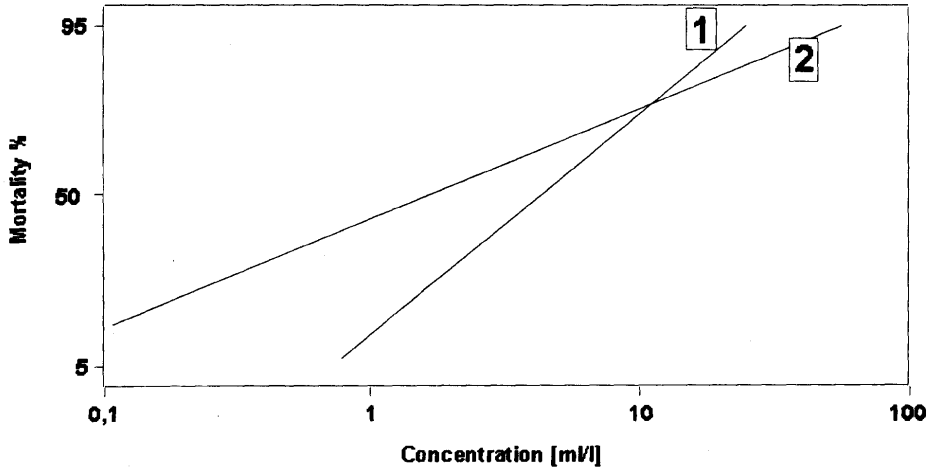

Figure 4. Regression lines obtained from studies on Lepidoptera caterpillars exposed to pine needles treated with Ecotech Pro 07.5 OF. (1) L2/L3 instars of nun moth caterpillars (assessment after 4 days), (2) L1/L2 instars of pine moth caterpillars (assessment after 4 days).

Results on the susceptibility of nun moth and pine moth larvae to Ecotech Pro 07.5 OF (Fig. 4) lead to the same conclusion. It may be concluded that larvae of nun moth and pine moth are equally susceptible to both Foray 48 B and Ecotech Pro. This is due to the strain specificity and low frequency of the use of other *B. thuringiensis* biopreparations against forest Lepidoptera in the past.

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The Control of the Nun Moth (*Lymantria monacha* L.) in Poland: A Comparison of Two Strategies

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ABSTRACT The nun moth *Lymantria monacha* is a defoliator of coniferous trees in many European countries. In Poland, its cyclical outbreaks occur in Scots pine and spruce stands. In the last half of the century, insecticides were used to control the nun moth on a total area of 7.3 million ha.

During the course of an outbreak in 1993 and 1994, two different control strategies were utilized and the efficacy of treatments and side effects of the insecticides were estimated.

In 1993, pyrethroids were used against II-IV instar of nun moth larvae in stands where >90% defoliation was expected. The efficacy of treatments, calculated as larval mortality, was estimated to be ca. 87 %. The infested area increased by 7 times in the following year.

In 1994, stomach insecticides Dimilin 480 SC and Foray 48 B were used against I-II instars in stands where the expected level of defoliation ranged from 30% to 90%. Spraying was carried out in the stands in which a complete defoliation was predicted as well as in those stands where the outbreak just began. The treatment efficacy, based on larval mortality, was 100% and the nun moth outbreak collapsed.

The numbers of beneficial and other non-target arthropods killed and collected on 1 m^2 drop cloths in the stands treated with pyrethroids, were approximately 10 times higher than in the stands treated with stomach insecticides.

FORESTS IN POLAND cover 8.7 million ha, which accounts for about 28% of the country's total area. The composition of the forests includes: Scots pine (*Pinus sylvestris*; 70.0%), Norway spruce (*Picea abies*; 6.0%), Silver fir (*Abies alba*; 2.5%), Others (1.0%), Oak, beech, birch and others (20.5%).

Scots pine defoliating insects are major economic pests of Poland's forest stands. The main defoliators are the pine beauty moth (*Panolis flammea*), pine lappet (*Dendrolimus pini*), pine looper (*Bupalus piniarius*), nun moth (*Lymantria monacha*), and Diprionid sawflies.

The nun moth occurs in Scots pine, spruce and larch stands. At very high population densities larvae attack both conifer and deciduous species.

In the second half of the century, six nun moth outbreaks were recorded in Poland, and aerial treatments with insecticides were applied over a total area of 7.3 million ha. The largest outbreak occurred in 1978-1984 (Fig. 1), and covered 3.7 million ha of pine and spruce stands. The outbreak peaked in 1982, when over 2.3 million ha were sprayed with contact pyrethroids (95% of the treated area) and the stomach insecticides diflubenzuron and *Bacillus thuringiensis* (5%).

Pages 108-115 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

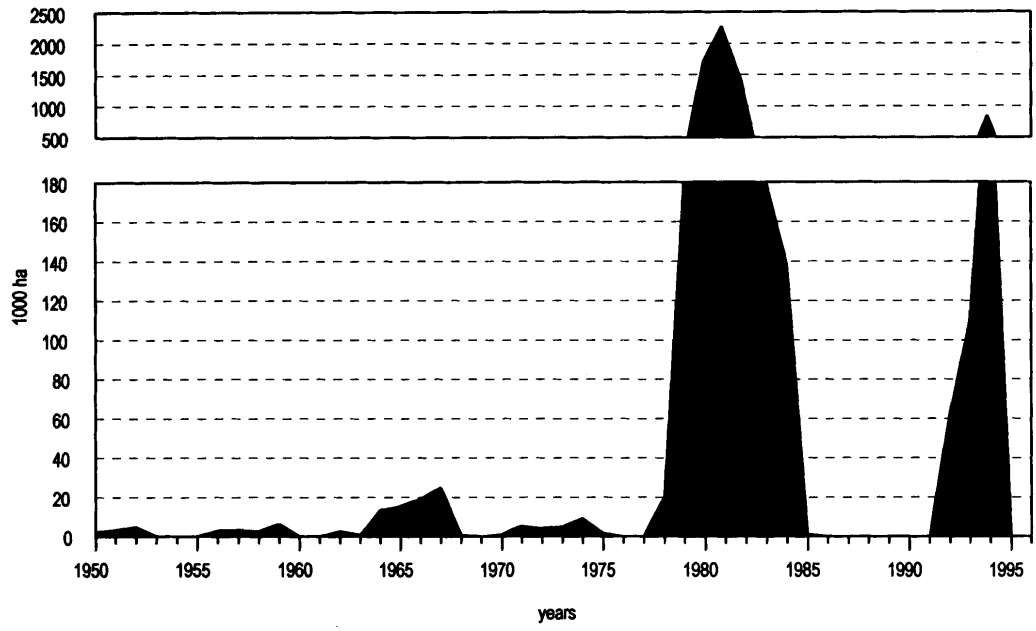

Figure 1. Aerial control treatments against the nun moth in Poland.

Table 1. Level of infestation with *Lymantria monacha* (females per tree)

Stand age (in years)	Warning numbers[1]	Numbers necessary to cause weak and/or moderate infestation (+ or ++)[2]	Critical numbers necessary to cause severe infestation (+++)[3]	
			Full foliage II and III class of quality site	Damaged foliage IV and V class of quality site
to 20	1	2 - 4	5	3
21-40	3	4 -19	20	10
41-60	6	7 - 39	40	20
61-80	10	11 - 59	60	35
81-100	15	16 - 79	80	50

[1]Warning numbers indicate a high probability of outbreak development.
[2]Numbers necessary to cause a weak or moderate infestation (+ or ++) with an estimated 30-90% stand defoliation.
[3]Critical numbers necessary to cause severe infestation (+++) with an estimated 90-100% stand defoliation.

The number of female moths observed on check trees during a swarm flight in July and August was used to forecast the infestation in the next year. Control treatments were recommended if the number of females observed on check trees indicated a possibility of complete (>90%) stand defoliation (Table 1).

The Nun Moth Control Treatments in 1993 and 1994

Since 1991, an increase in the density of nun moth populations was observed and in 1992, the infested area covered 160,000 ha of Scots pine stands. Control treatments were

carried out on an area of 62,600 ha using pyrethroids. In spite of the satisfactory efficacy of the spraying, the forested area infested with the nun moth increased in the following year.

In 1993, control treatments were carried out using the contact pyrethroids Sumi-Alpha 005 UL, Decis 2,5 EC, Fastac 10 EC and stomach insecticides Dimilin 480 SC and *B. thuringiensis* (Fig. 2). The treatments were performed against II-IV instar nun moth larvae. An aircraft equipped with Micronair AU 3000 nozzles sprayed 110,000 ha of Scots pine stands in which heavy (>90%) defoliation was expected.

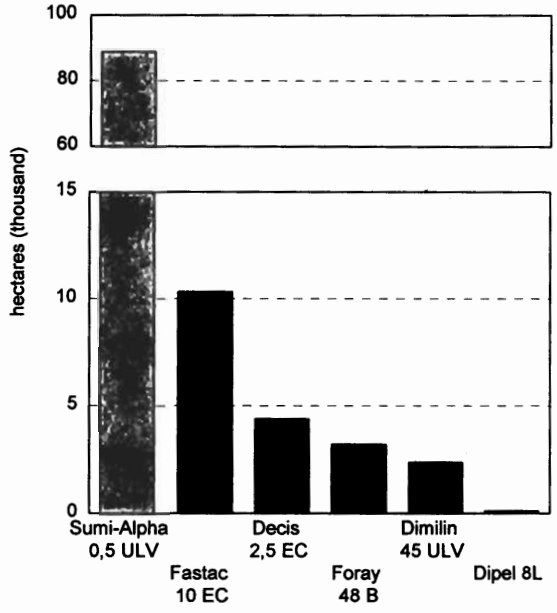

Figure 2. Insecticides used in nun moth control treatments in 1993.

In 1994, a new control strategy was developed to suppress pest outbreak. A project "The integrated control of nun moth in Polish forests," financed by Polish and foreign funds, was implemented. The new approach was aimed at reducing nun moth larval populations through the use of stomach insecticides. The use of pyrethroids was reduced to 10% (Fig. 3). The 757,000 ha of infested Scots pine stands were sprayed with an aircraft equipped with Micronair AU 5000 nozzles. The level of infestation based on predicted defoliation ranged from 30% to 90% and included stands where the nun moth outbreak was just beginning. The treatments were applied at the beginning of May, as soon as the nun moth larvae began hatching.

The Efficacy of Control Treatments in 1993 and 1994

Nun moth mortality following treatments was estimated in 1993 and 1994 using linen 1 m^2 cloths that were placed beneath check trees (420 cloths in 1993, and 450 in 1994). The number of dead nun moth larvae dropping from the crowns was counted over a period of 7 days in the stands treated with pyrethroids, and for a few weeks in those stands treated with stomach insecticides. Trees were then cut and placed on large linen sheets. The remaining larvae that were stuck to needles were collected and percent mortality was calculated by

multiplying the number of the larvae dropped/m^2 by the area of crown projection, and including the number of both living and dead larvae found in the crown.

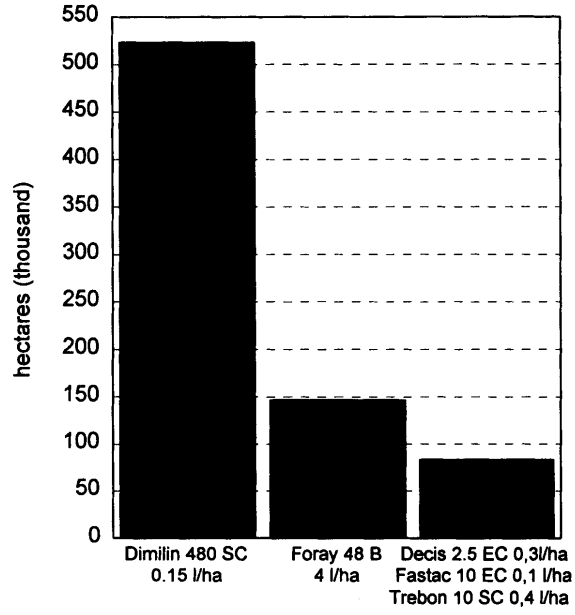

Figure 3. Insecticides used in nun moth control treatments in 1994.

In 1993, the mortality of the nun moth was calculated 1-2 weeks following treatment. The average number of dead larvae dropped per m^2 linen cloth was determined after each treatment (Fig. 4). In the stands treated with Dimilin 480 SC. an average of 18 larvae were found per m^2 cloth, and in the stands treated with Fastac 10 EC, the average number of dead larvae was more than 80. Nun moth larval mortality ranged from 17% to 100% (Fig. 5) with an average mortality nearing 87%. However, the infested area increased 7 fold in the following year.

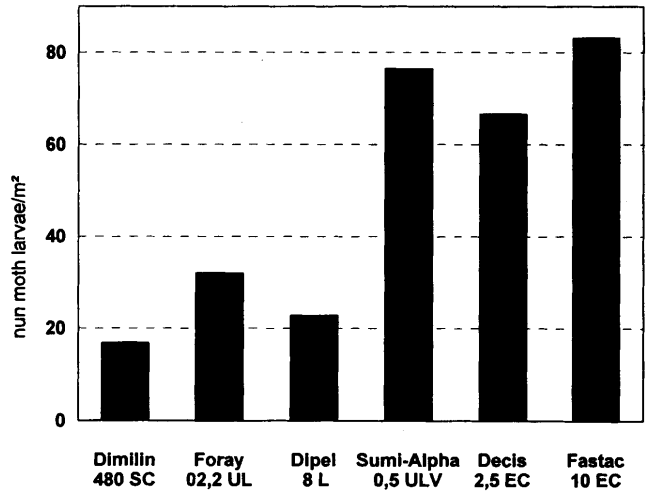

Figure 4. The mean number of nun moth larvae/m^2 in 1993.

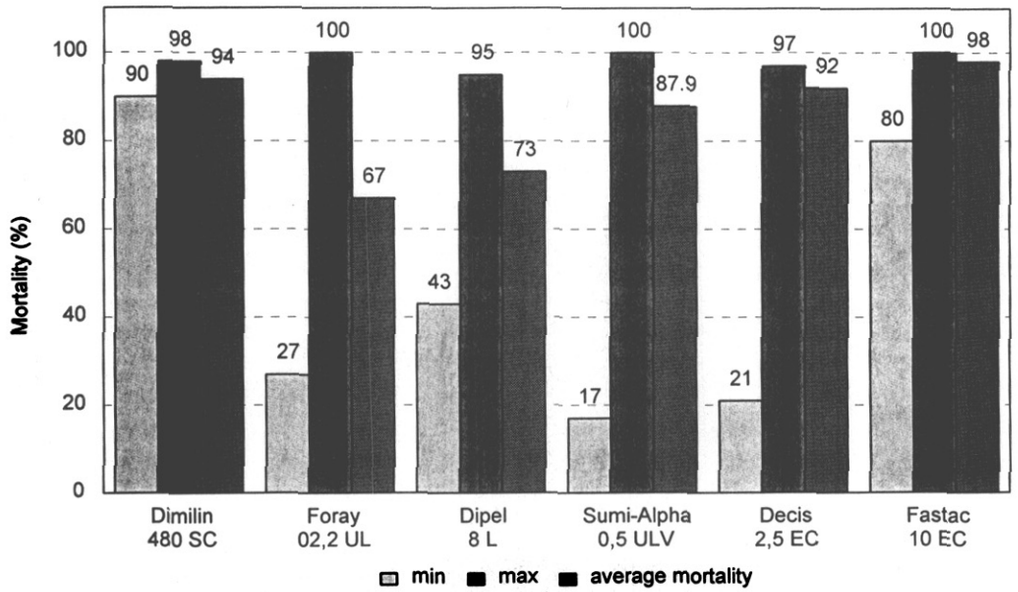

Figure 5. Mortality of nun moth larvae in 1993.

In 1994, the mortality of the nun moth on check trees was calculated 2 and 4 weeks following the treatment. The average number of dead larvae collected per m^2 ranged from 37 in the stands treated with Foray 48 B to 250 in the stands treated with Decis 2,5 EC (Fig. 6). Ultimately, the mortality approximated 100% (Fig. 7) and resulted in the cessation of the outbreak.

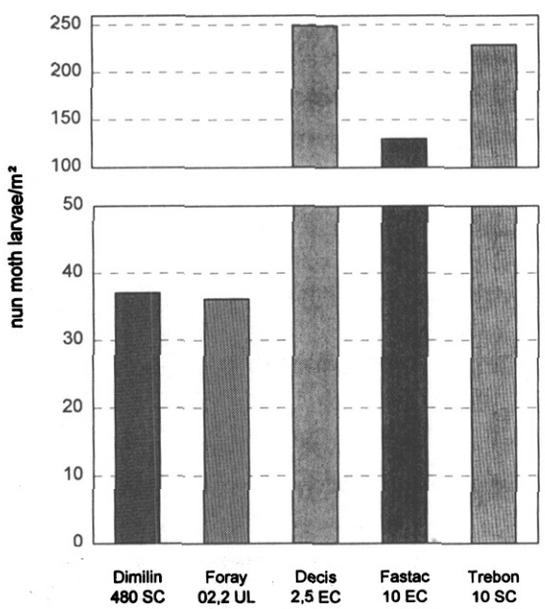

Figure 6. The mean number of nun moth larvae/m² in 1994.

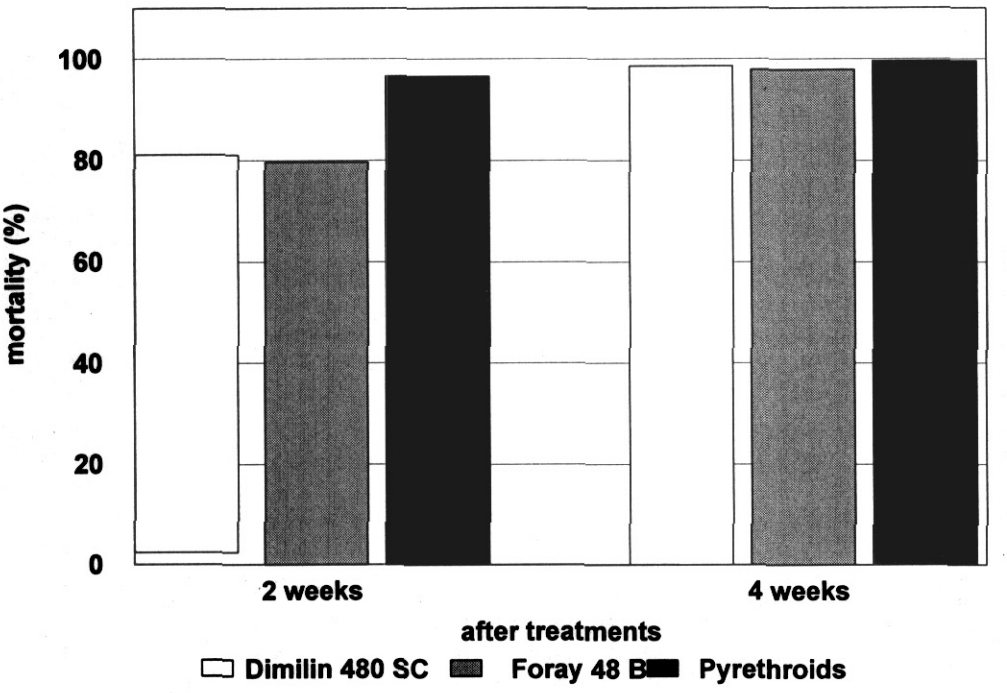

Figure 7. Mortality of nun moth larvae in 1994.

The additional factor that favored high nun moth mortality in 1994 was the poor synchrony between the development of pine and nun moth larvae caused by above normal temperatures in April and below normal temperatures in May and June (Table 2).

The early hatching of larvae and delayed development of young pine shoots and male inflourescences resulted in a continued deficit of suitable food for young larvae; this may have caused the reduced viability observed in the nun moth populations.

Mortality of Non Target Arthropods

An estimation of the environmental impact of the control treatments was carried out in 1993 and 1994. The impact of both stomach and contact insecticides on parasitoids, predators, and other non-target insects and spiders was assessed as the number of dead arthropods found after the treatment on 1 m^2 linen cloth placed beneath the crowns of 420 (1993) and 450 (1994) check trees.

The average numbers of dead beneficial and other non-target arthropods collected per m^2 in the stands treated with pyrethroids were approximately 10 times higher (Figs. 8-9) than in the stands treated with *B. thuringiensis*, diflubenzuron, and in the control plots.

Table 2. Thermal characteristics of successive decades of April (Ap), May and June in 1993 and 1994 (acc. IMiGW)

Meteorological station	1993							1994						
	Month													
	Ap	May			June			Ap	May			June		
	Decade													
	3	1	2	3	1	2	3	3	1	2	3	1	2	3
Bialystok	A	A	A	a	N	B	B	A	b	A	B	b	B	N
Chojnice	A	A	A	A	a	b	B	A	N	a	B	b	B	a
Gdansk	A	A	A	A	a	b	B	A	b	a	b	b	B	N
Katowice	A	A	A	A	A	b	B	A	b	A	b	b	B	A
Kielce	A	A	A	a	a	b	B	A	b	A	b	b	b	a
Koszalin	A	A	A	A	N	N	B	A	a	a	b	b	B	a
Kraków	A	A	A	a	A	b	B	A	b	A	b	b	B	A
Lublin	A	A	A	a	a	b	B	A	B	A	B	B	B	a
Lodz	A	A	A	a	A	b	B	A	N	A	B	b	B	A
Olsztyn	A	A	A	A	N	b	B	A	b	A	B	b	B	N
Opole	A	A	A	a	A	b	B	A	b	A	b	N	B	A
Poznan	A	A	A	a	a	B	B	A	N	A	B	b	B	a
Rzeszów	A	A	A	a	a	b	B	A	b	A	b	b	b	A
Szczecin	A	A	A	a	a	b	B	A	a	A	b	b	B	a
Warszawa	A	A	A	a	a	b	B	A	b	A	B	b	B	a
Wroclaw	A	A	A	a	A	b	B	A	N	A	b	b	B	A
Zielona Góra	A	A	A	a	A	B	B	A	N	A	b	b	B	A

A	much above normal
a	above normal
N	normal
B	much below normal
b	below normal

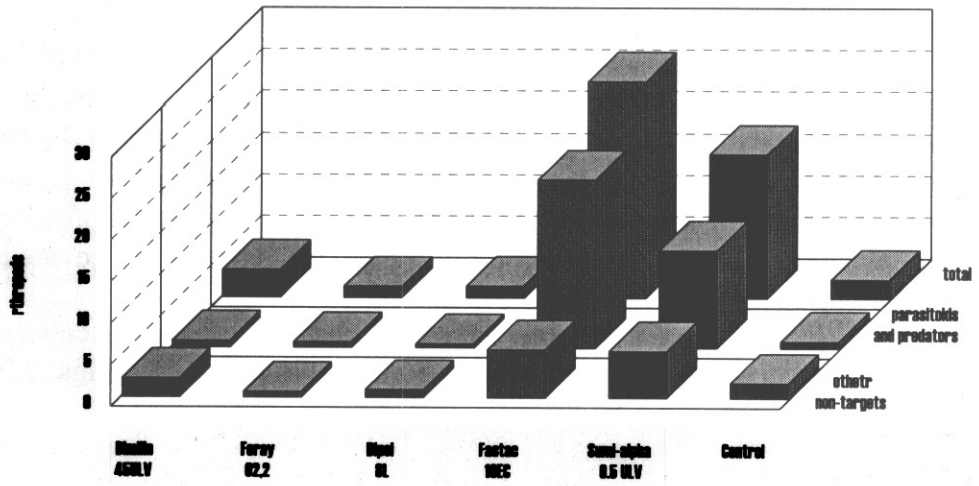

Figure 8. Average number of beneficial and indifferent arthropods dropped per m^2 in treated stands in 1993.

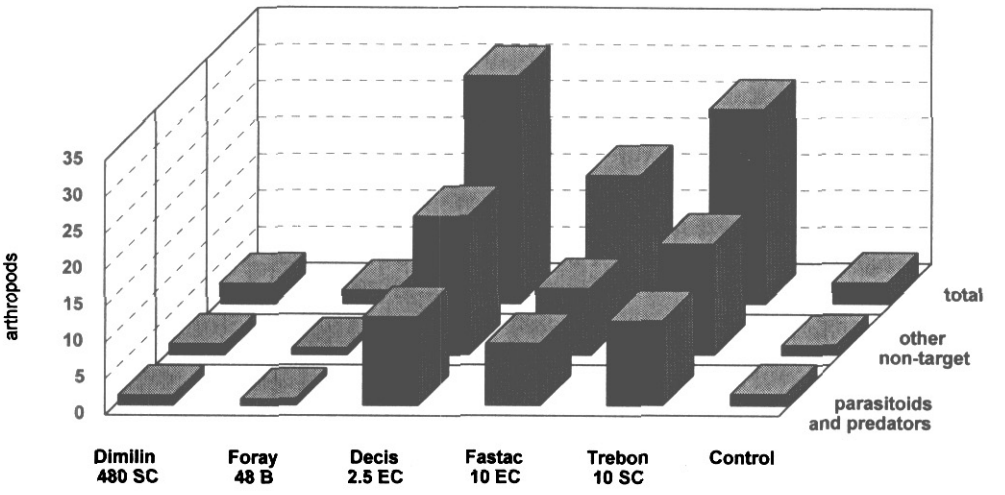

Figure 9. Average number of dead beneficial and other non-target arthropods collected per m^2 in treated stands in 1993.

Conclusions

In 1993, the II-IV instar nun moth larvae were controlled with pyrethroids in stands in which the population density of the pest was expected to cause >90% defoliation. However, the area of infested stands increased in the following year by 7 times.

The use of stomach insecticides Dimilin 480 SC and Foray 48 B against newly hatched larvae in 1994, not only in the stands in which complete defoliation was expected, but also in those stands with lower density nun moth populations, caused high mortality of the larvae and resulted in the cessation of the outbreak.

The comparison of the side effect of the insecticides used here showed that the mortality of parasitoids, predators and economically indifferent arthropods in the stands treated with pyrethroids was 10 times higher than in the stands treated with stomach insecticides and in untreated stands.

The Effect of Pesticides Applied Aerially to Forest Stands on Four Species of Native Hymenopterous Parasitoids

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ABSTRACT Studies were conducted on the Ostrow Mazowiecka Forest District in central Poland to assess the impact of aerially spraying four pesticides (Decis 2.5 EC, Trebon 10 EC, Foray 48B, and Dimilin 480 SC) against the nun moth (*Lymantria monacha* L.) on the dynamics of four species of hymenopterous parasitoids that are common inhabitants in Scots pine forests. Results are discussed in relationship to the behavior and bionomics of the individual parasitoid species and to their hosts.

THE SUPERFAMILY *ICHNEUMONOIDEA* is one of the largest groups of insect parasitoids, and contains many species that attack a wide variety of insect hosts and that possess many behavioral adaptations. These attributes contribute to their importance in the biological control of many forest insect pest species. Outbreaks of many forest defoliators occur in Poland, requiring at times the application of pesticides to thousands of hectares in order to prevent defoliation and tree mortality. There is concern about the effect of aerially-applied pesticides on forest ecosystems, and specifically on non-target species such as native parasitoids. Consequently, the Department of Forest Protection, Forest Research Institute, initiated studies to determine the impact of pesticide treatments used against the nun moth, *Lymantria monacha* in Scots pine (*Pinus sylvestris*) stands on select species of parasitoids.

Methods

Studies were conducted in 1994 and 1995 in the Ostrow Mazowiecka forest district in central Poland. Four treatment plots and a control plot were established in a 40 year old even-aged stand of Scots pine. Four pesticides were applied aerially on May 22, 1994, using ULV equipment (Micronair AU 5000): Trebon 10 EC, Decis 2.5 EC, Foray 48B, and Dimilin 480 SC.

In order to estimate the mortality after treatments five, 1 m^2 linen drop cloths were placed in each plot under the average trees. The cloths were checked on six successive days post-treatment in the plots sprayed with contact pesticides Trebon 10 EC and Decis 2.5 EC. In the plots sprayed with Foray 48B and Dimilin 480 SC, the cloths were checked every third day post-treatment over a period of 2 weeks.

Changes in the abundance of native parasitoids were estimated by deploying Moericke's yellow pan traps (6/plot) in the middle of tree crowns at 5 to 8 m above ground depending on tree height. The traps were checked every 2 weeks during the period of May 5 to October 20 in both 1994 and 1995.

Pages 116-121 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

Four species of parasitoids were chosen for analysis based on their differing behavior, biology, and effect on their hosts. *Agrypon flaveolatum* (Grav.), *Idiogramma euryops* (Foerst.), and *Lestricus secalis* (L.) are koinobionts, which are proovigenic and have a relatively short Spring flight period (Askew and Shaw 1988). Two species of ichneumonids that are closely related biologically, *Pimpla turionellae* (L.) and *P. contemplator* (Mueller) were combined because of questions about their taxonomy. These species are characteristic of idiobionts, which are synovigenic and have a prolonged flight period.

Results and Discussion

The analysis of collections from the linen drop cloths indicate that none of the pesticide treatments had an effect on any of the chosen species of parasitoids. Because the treatments were applied early in the morning prior to the period of activity of adult parasitoids, there was very low mortality caused even by the contact pesticides Trebon 10 EC and Decis 2.5 EC. This was further confirmed by the large number of ichneumonids that were recovered in the plots (Fig. 1).

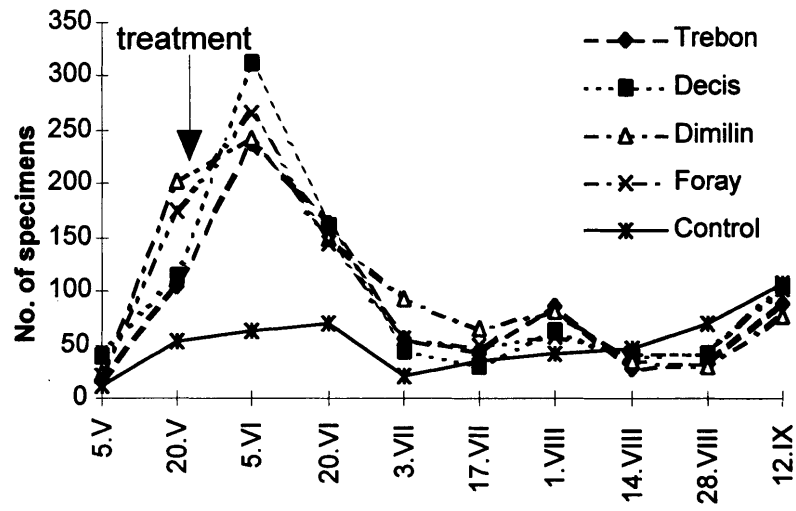

Figure 1. Abundance of *Ichneumonidae* collected in Moericke traps, Ostrów Mazowiecka forest district, 1994.

Based on collections from the pan traps, the pesticide treatments had either no effect or a positive effect on populations of the koinobiont species *A. flaveolatum, I. euryops,* and *L. secalis*; the idiobiont species (*Pimpla*) declined in 1995, the year after the plots were treated. The results are discussed in relationship to the behaviors of these species and the dynamics of their host populations.

Females of the koinobiont species are proovigenic, that is, they contain completely developed eggs at the time they emerge, and therefore do not require supplementary feeding prior to parasitizing their host larvae. Therefore, they spend most of their adult life searching for suitable hosts in which to oviposit. This contributes in part to their relatively short flight period (Figs. 2, 3, 4). In addition, they are univoltine and, thus, well adapted to their individual host species. *A. flaveolatum* is a Palearctic species in the subfamily anomaloninae

and is known to parasitize larvae of many lepidopteran species, especially within the families Tortricidae, Noctuidae and Geometridae. This species is an important natural enemy of the winter moth, *Operophtera brumata* L., and was introduced successfully into North America to control this forest pest (Barrons 1989). Females of *A. flaveolatum* deposit their eggs into early instar larvae of their hosts whereupon the parasitoid larva hatches, completes its development within the host, and then emerges from the host pupa. Although populations of free living host larvae might be reduced by the pesticide treatments, thus having a negative effect on the parasitoid, this was not observed since numbers of *A. flaveolatum* recorded from traps were similar in both years on all plots except the plot treated with Dimilin, where a significant increase occurred in 1995 (Fig. 2). Since many of the geometrid larvae were in the late instars at the time of spraying, it is possible that *A. flaveolatum* larvae were able to complete their development successfully prior to the death of their hosts. It has been reported that diflubenzuron, the active ingredient in Dimilin, can cause an increase in progeny of some parasitoids (Khoo et al. 1985). This could possibly account in part for the high abundance of *A. flaveolatum* in the Dimilin plot in 1995. However, additional research studies are needed to confirm that this phenomenon occurs in this species.

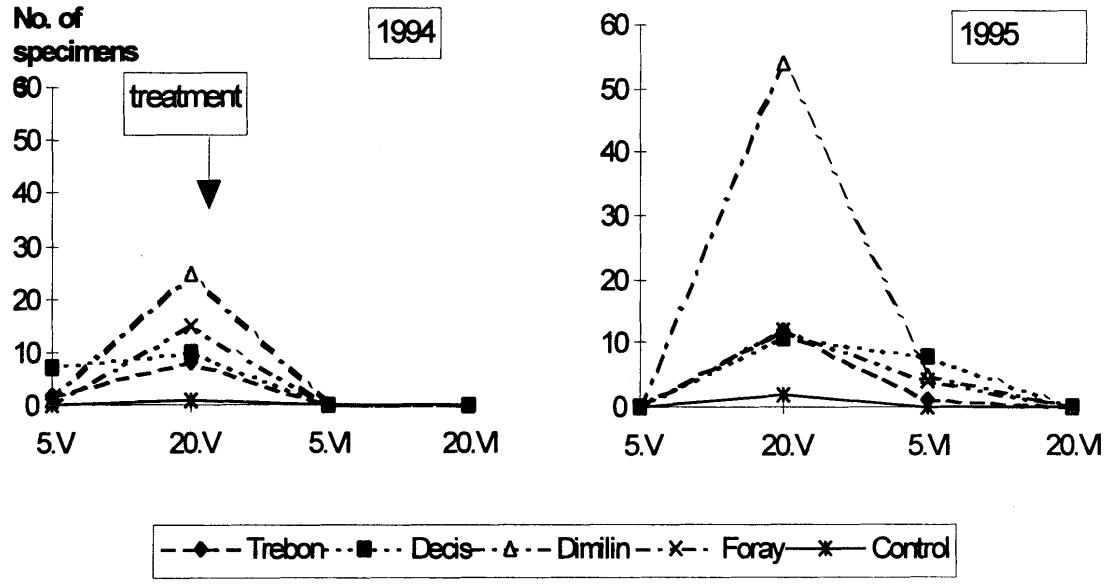

Figure 2. Abundance of Agrypon flaveolatum (Grav.) collected in Moericke traps, Ostrów Mazowiecka forest district, 1994-95.

Idiogramma euryops is a small tryphonine that is known to parasitize xyelid (Hymenoptera: Xyelidae) larvae that live concealed in male inflorescens of *Pinus* sp. Although the pesticide treatments should not have directly affected the xyelid hosts of this parasitoid, the treatments may have effectively eliminated the nun moth first instars that also feed upon and destroy pine inflorescens, and thus compete with the xyelid larvae for suitable habitats. Reduction in nun moth populations may have benefited both from the xyelid population and *I. euryops* and, thus, account for the increased abundance of *I. euryops* that was recorded in 1995 (Fig. 3).

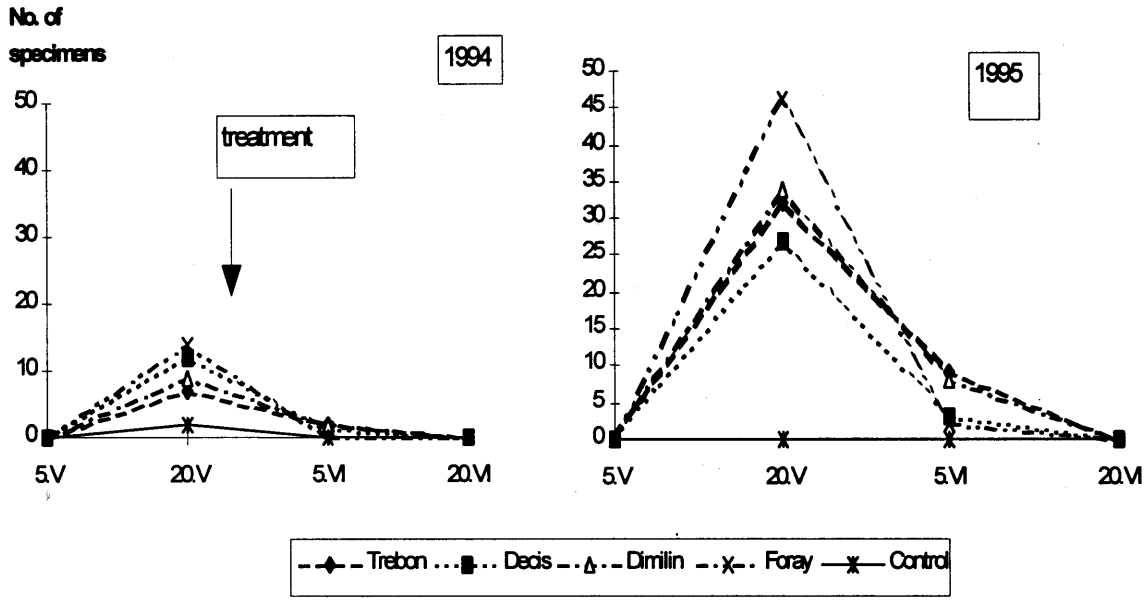

Figure 3. Abundance of *Idiogramma euryops* (Foerst.) collected in Moericke traps, Ostrów Mazowiecka forest district, 1994-95.

The other koinobiont, *L. secalis*, is a small braconid wasp in the small world-wide subfamily Cenocoeliinae, that parasitizes small wood-boring beetle larvae (Shaw and Huddleston 1991). In our plots, we recovered this species from *Pogonocherus decoratus* Fairm. and *P. fasciculatus* (DeGeer) (Coleoptera: Cerambycidae), whose larvae live in dead and dying twigs of conifers. Therefore, these hosts should not be affected by treatments of aerially applied pesticides. However, the abundance of populations of *P. decoratus* and *P. fasciculatus* might be affected more strongly by outbreak nun moth populations that cause death and weakening of *P. sylvestris* and thus provide more host material for the cerambycids. In fact, the abundance of *L. secalis* was lower in the control plot (Fig. 4) where nun moth populations also were low (Glowacka 1994).

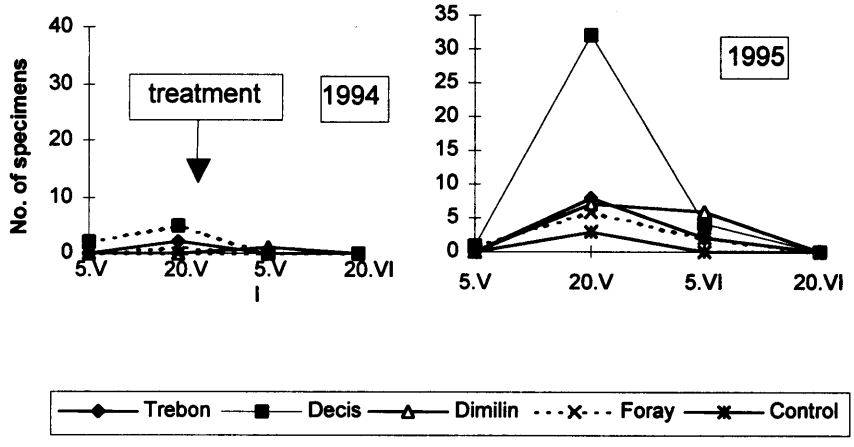

Figure 4. Abundance of *Lestricus secalis* (L.) collected in Moericke traps, Ostrów Mazowiecka forest district, 1994-95.

Pimpla turionellae and *P. contemplator* are idiobionts, which are synovigenic. The adult females are obligated to take supplementary food, and therefore, the time required for completion of egg development is prolonged. In addition, both species are bivoltine with peaks of abundance occurring at the beginning of June and toward the end of July (Fig. 5). Both species are polyphagous and parasitize small- and medium-sized pupae of many species of Lepidoptera in the families Geometridae, Tortricidae, Noctuidae, and Lymantriidae (Fitton et al. 1988). The abundance of *Pimpla* sp. was not affected by the treatments in 1994, however significant reductions were observed in 1995. These reductions apparently were caused by significant decreases in the host populations caused by the pesticide treatments.

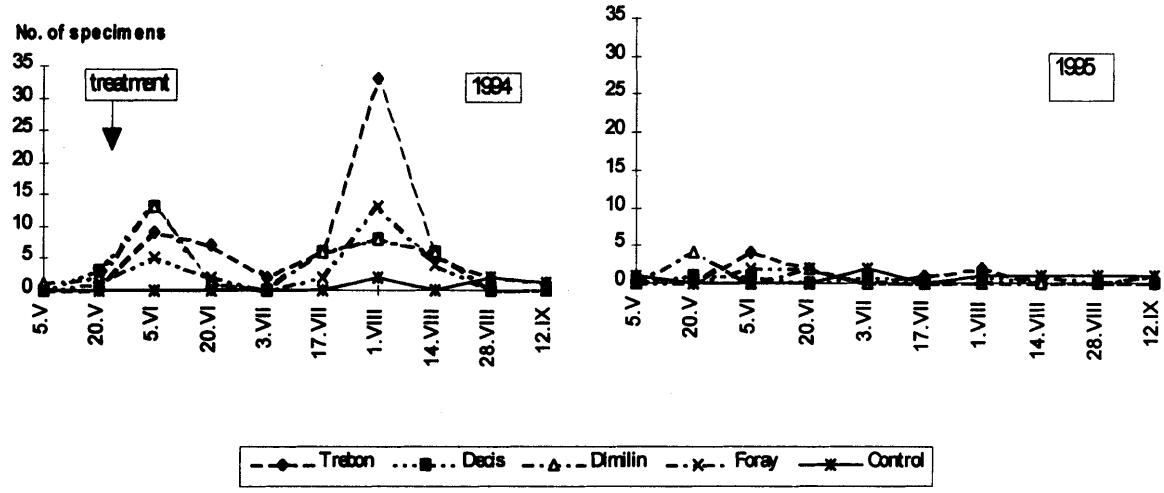

Figure 5. Abundance of Pimpla sp. collected in Moericke traps, Ostrów Mazowiecka forest district, 1994-95.

Although aerial application of pesticides to control forest defoliators may have many direct and indirect effects on populations of natural enemies, these effects will vary depending on the behavior and dynamics of both hosts and parasitoids. Adult hymenopteran parasitoid species that are active in tree crowns may be particularly susceptible to contact pesticides such as Trebon 10 EC and Decis 2.5 EC. However, their susceptibility to these products varies depending on their period of flight activity and the timing of the pesticide application. The abundance of the four parasitoid species in this study probably was affected more by the abundance of their host populations, which in turn varied according to their distribution in the forest and their susceptibility to the pesticide treatments.

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Evaluating the Impact of Insect Communities on Pine Caterpillar Density under Different Stand Conditions

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ABSTRACT Natural enemies play an important role in inhibiting the population dynamics of the pine caterpillar, but their roles are difficult to incorporate into systematic management models. This paper attempts to use selected forest stand factors to substitute for the effects of natural enemies, for use in management models. From studies that include more than 200 plots under different stand conditions in Qu County, Changshan County and Longyou County of Zhejiang Province, 16 predominant species groups from the insect community were selected, and the canonical correlation coefficient between the insect diversity index, number of insect species, number of predominant insect species and stand factors was determined. The results indicated that both canopy density and vegetation condition are the key stand factors that characterize the dynamics of the insect community. The forest area was classified into four types according to variation in canopy and vegetation conditions using systematic cluster analysis. There were significant differences in the insect diversity index, number of insect species, number of predominant insect species and population density of the pine caterpillar among the four types. Regression models between pine caterpillar density and forest factors, including canopy density and vegetation cover were developed for each type of forest. The correlation coefficients of the four regression models were rather high (r=0.924-0.964). This indicates that the classification of forest stands is reasonable and stand factors may be used to simulate the effect of natural enemies in the field.

THE PINE CATERPILLAR, *Dendrolimus punctatus* (W.), is a serious defoliating pest of masson pine forests in the southern part of China and occurs in 15 provinces and regions. The population dynamics of the pine caterpillar are a major component of the integrated management system of this insect. Natural enemies play an important role in inhibiting the population density of the pine caterpillar, but past research work has centered on natural enemy classification, individual biology and evaluating their effect by constructing life tables. (Wu 1979, Cheng 1984, Wang1984, Chai and Li 1985, Zha 1988, Chen and others 1990). The results were difficult to use in systematic management models. Past research work has shown that in the field, forest plant and insect communities interact with each other (Bach 1980, Frances 1982, Lawton 1978, MacArthur 1964, Southwood and others 1979). Stand factors affect insect communities, which further affect pine caterpillar population dynamics. In this paper, we focus on the impact of the insect community on pine caterpillar density with the goal of identifying forest stand factors that simulate the effect of insect communities on this pest and are easy to use in management models.

Pages 122-128 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

Materials and Methods

Field work was conducted between 1992 to 1994 in masson pine forests located in Longyou County, Qu County and Changshan County of Zhejiang Province. Most of the pine forests are pure pine and distributed on hillsides. Stand ages ranged from 5 to 20 years, tree heights ranged from 2 to 6 meters, and diameter at breast height ranged from 5-12 centimeters. Canopy densities varied from 0.3 to 0.8, and vegetative cover varied greatly, with herbs or shrubs being predominant. From 1992 to 1994, ground surveys were conducted on 222 experimental plots six times per year.

Survey Period. The survey time frame encompassed the late larval and pupal stages following overwintering, the early larval, late larval, and pupal stages of the first generation, and larval stages of the second generation before overwintering.

Sampling Methods. Two sampling methods were used. The first involved covering a pine branch sample with a net, cutting the branch with long-stem scissors, then counting the number and species of insects. Two branches were cut on each of 50 trees for a total of 100 samples per plot. A total of 70 groups of insects was found using this method. Another method involved sweeping insects on the ground vegetation with a sweepnet.

Factors of Investigation. Factors of investigation included pine caterpillar density; tree age and canopy density; elevation, slope and aspect; and vegetative conditions, including the height of shrubs and herbs, percent herb and shrub cover, total percent cover and average percent cover.

Data Analysis. Predominant Species or Species Group Determination: The selection criteria for the predominant species or species groups included: the individual number of insects per sampling unit more than 5 and the value of the correlation coefficient between the density of predominant species groups and density of the pine caterpillar. Most predominant species or species groups are natural enemies of the pine caterpillar. According to the above selection criteria, 16 species or species groups were selected as predominant species. These include: Pantatomidae, Reduviidae, Coreidae, Carabidae, Meloidae, Aphididae, Formicidae, Chalcididae, Ichneumomidae, Braconidae, Coccinellidae, spider, Lymantriidae, Chrysopidae, Syrphidae and Alydidae.

Expression of the Function of Predominant Species Groups. The individual number of insects is denoted by N, the number of species or species groups is denoted by S and the diversity index is denoted by H. These variables were chosen to represent the dynamics of the predominant species or species groups. H was calculated using the Gini-Simpson method. The correlation coefficients between N, S, H and the density of the pine caterpillar, D, were calculated.

Table 1. Correlation coefficients between N, S, H and D

R	Min	Max	Average
N-D	0.32	0.83	0.51
S-D	0.23	0.63	0.43
H-D	0.28	0.69	0.45

The results showed that all of the coefficients are higher than the critical point at the 5% (0.25) significance level (Table 1). Therefore, using N, S and H to describe the impact of the insect community on pine caterpillar populations was suitable.

The Selection of Key Stand Factors. The canonical correlation analysis method was adopted to evaluate the effect of stand factors on the insect community. This method can delete relative effects among the factors and analyze the relative of each factor against N, S and H. The canonical correlation coefficient between N, S and H of the 16 predominant species groups and each stand factor was analyzed. The results showed that canopy density and vegetation condition were the principal stand factors for characterizing the dynamics of the insect community. The results are shown in Table 2.

Table 2. Key stand factors

County	Number of Factors	Key Stand Factors
Qu	4	canopy density, shrub coverage, herb height, total coverage
Longyou	3	canopy density, herb height, total coverage
Changshan	3	canopy density, shrub coverage, shrub height

Classification of Forest Stands. The forest area was classified into four types, denoted as type I, II, III, and IV, by using systematic cluster analysis according to the above conditions. The two-dimensional presentation of the classification is shown in Figures 1 and 2.

The results of the classification for the three counties were consistent. In order to see if the classification was reasonable, we first compared the values of N, S, H and D of forest type (I, II) (i.e., forest type I and type II together) and forest type (III, IV). The calculation results indicated that there were significant differences between type (I, II) and (III, IV) forests (Table 3). Then, on the basis of the first step, we compared the difference between type I and II and between type III and IV. The results indicated that there were also significant differences between type I and II as well as between type III and IV (Table 4). These results demonstrated that the classification of forest stands was reasonable, because there was a different inhibition effect on the pine caterpillar among the four types of forest. Furthermore, this effect among the four types of forest could be expressed as function of Nt, St, Ht and D(t).

Modelling Community Indices and Stand Factors. By denoting $Y_t = D(t+1)/D(t)$, that is, the ratio of the population density of the pine caterpillar at time t+1 and time t, then the linear regression models of Y_t on N_t, S_t, H_t and D(t) are:

Forest Type I: $Y_t=5.356-1.589N_t+2.280S_t-5.457H_t$ R=0.701

Forest Type II: $Y_t=-3.968+1.407N_t-4.446S_t-22.225H_t$ R=0.981

Forest Type III: $Y_t=2.992-0.155N_t-0.385S_t+1.375H_t$ R=0.771

Forest Type IV: $Y_t=4.092+0.021N_t-0.493S_t+2.261H_t$ R=0.962 .

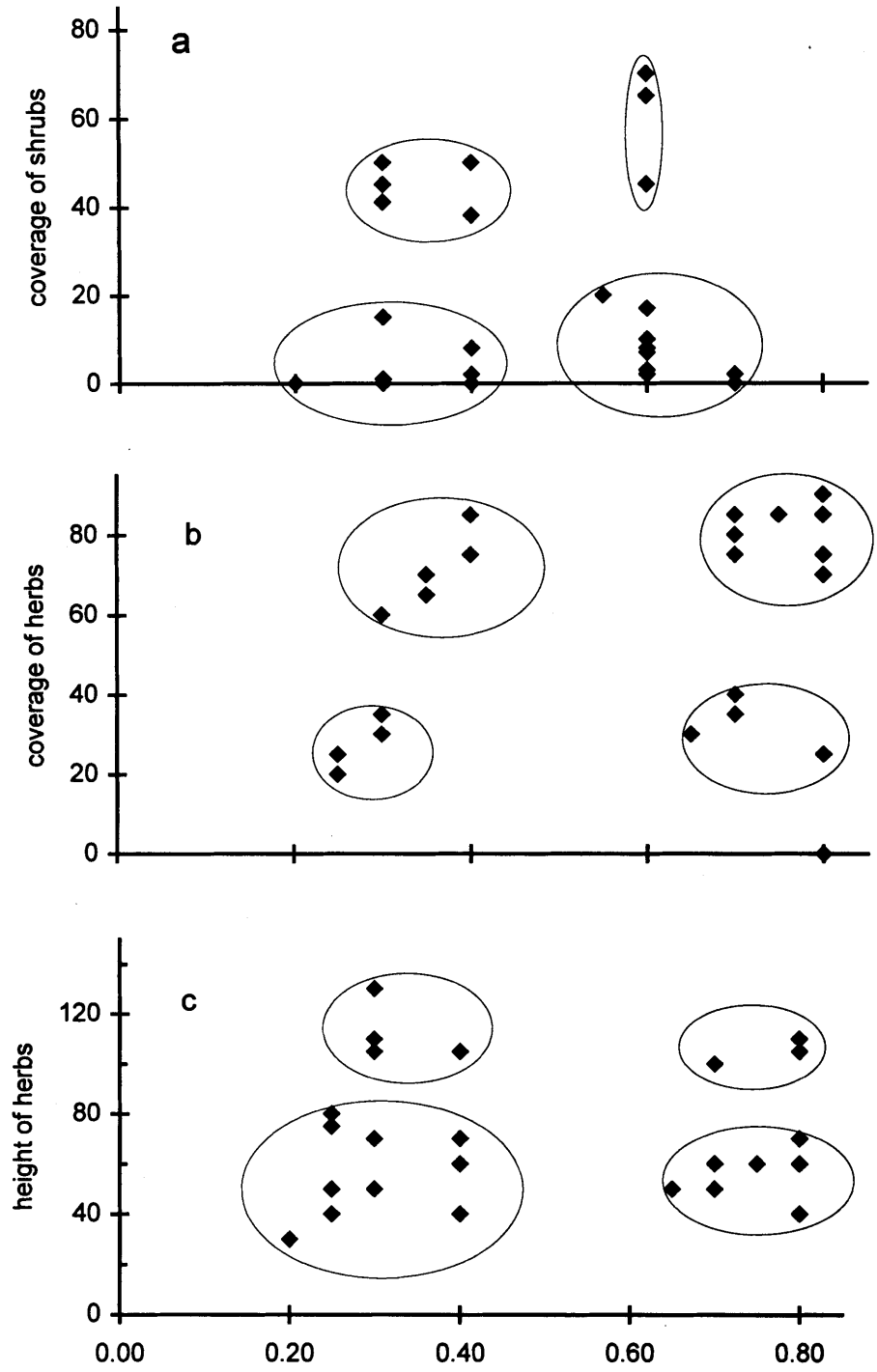

Figure 1. Classification for forest stand conditions. a: Changshan County, b: Longyou County, c: Qu County. Horizontal axis for all: canopy density.

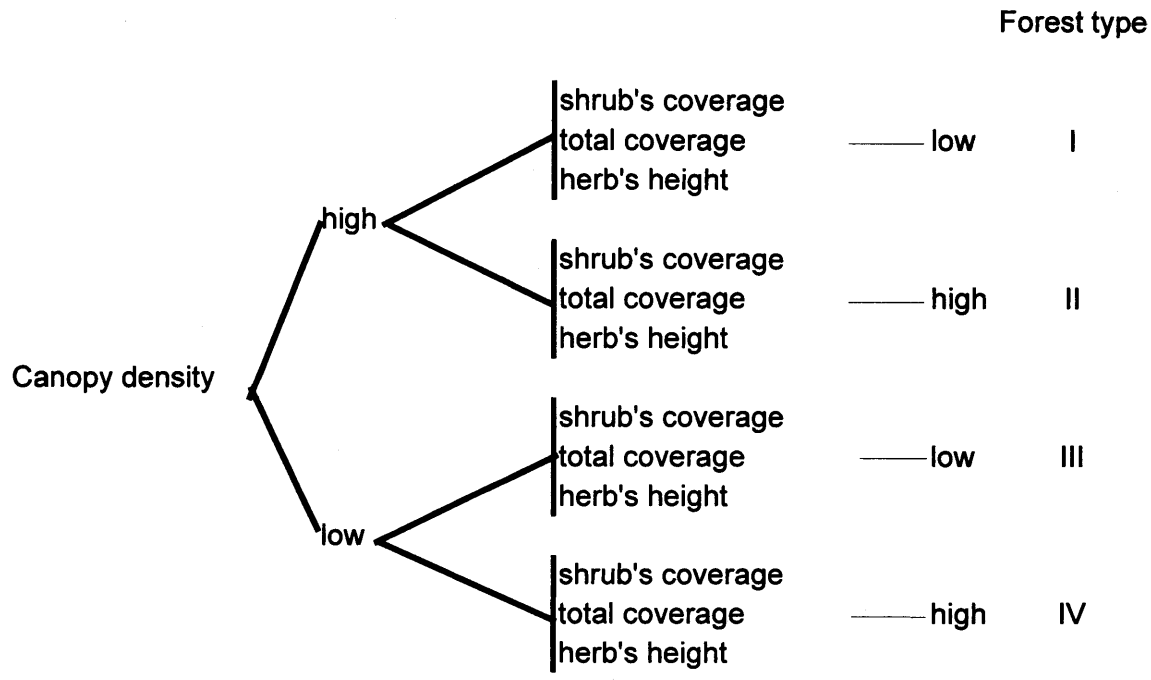

Figure 2. Result of classification for forest stand conditions.

Table 3. Comparison between type (I, II) and type (III, IV)

Statistics	N		S		H		D	
	(I, II)	(III, IV)	(I, II)	(III, IV)	(I, II)	(III, IV)	(I, II)	(III, IV)
mean	9.22	6.90	2.50	2.00	1.52	0.74	13.97	10.60
sample number	78	78	78	78	79	79	66	71
student t	14.49		3.12		4.91		19.76	
prob<0.01	yes		yes		yes		yes	

Table 4a. Comparison between forest type I and forest type II

Statistics	N		S		H		D	
	I	II	I	II	I	II	I	II
mean	9.92	8.59	2.33	2.61	1.83	1.46	15.93	12.20
sample number	37	41	36	41	37	41	31	35
student t	5.88***		-1.66**		1.25*		14.96***	

*** p<0.01, ** p<0.05, * p<0.10

Table 4b. Comparison between forest type III and forest type IV

Statistics	N		S		H		D	
	III	IV	III	IV	III	IV	III	IV
mean	6.33	7.38	1.81	2.17	0.61	0.87	12.14	9.25
sample number	36	42	36	42	36	42	33	38
student t	-4.61***		-1.59**		-1.25*		12.14***	

*** $p<0.01$, ** $p<0.05$, * $p<0.10$

The correlation coefficients between N, S, H and the population density of the pine caterpillar are large for all four formulas. The formulas above showed that the effects of N, S and H on population densities were different in different forest types; the negative and positive regression coefficients indicated that in an ecosystem, diversity may or may not be positively related to the stability of that ecosystem.

The relationship between the diversity and stability of an ecosystem is complex. It can be explained by structure analysis. There are serial connection and parallel connection structures. There also may be positive relations in parallel connection structures due to the increase of redundancy, and this may increase system stability. On the other hand, in series connection structures, diversity cannot increase the stability of the system (Liu 1991). Unfortunately, under natural conditions, N, S and H are not easy to survey; however, forest stand factors can be relatively easy to investigate., Actually, Y_t can be expressed as Y(t)=f (forest stand factors) =f (ybd, gh, gc, ch, zc) in which ybd is canopy density, gh is shrub layer height, gc is shrub coverage, ch is herb layer height and zh is total coverage. The stepwise regression results are listed below:

Forest Type I:

Y_t=3.620+7.933ybd-0.091gh-0.021gc+0.020ch-0.159zc-0.134D(t) R=0.961

Forest Type II:

Y_t=-29.148+0.346gh+63.011ybd-0.677ch+0.567zc R=0.924

Forest Type III:

Y_t=2.318+2.171ybd+0.006ch-0.089D(t) R=0.964

Forest Type IV:

Y_t=4.199-0.128ybd-0.097D(t) R=0.957

Obviously, the correlation coefficient between Y_t and the stand factors is higher than the correlation coefficient between Y_t and N, S and H of the insect community. The variable D(t) was left in the regression models, because the variation of insect density is related to former insect density.

Conclusion

The effect of the insect community on the pine caterpillar plays an important role in pine caterpillar population dynamics. Usually, the dynamics of an insect community can be described by insect diversity, the number of individuals, the number of species and other indices. Unfortunately, it is difficult to quantify these community indices. On the other hand, forest stand factors, such as canopy, vegetation coverage, etc., can be easily surveyed. Thus,

we tried to find a way to use forest stand factors instead of insect community indices to express the impact of the insect community on the pine caterpillar. We first used the 16 predominant species or species groups from previous studies to identify key forest stand factors, then used these key stand factors to classify forest stand, and finally, established relationships between the variation in pine caterpillar density and stand factors. The results showed that canopy density and vegetation condition were the key stand factors for characterizing the dynamics of the insect community.

Forest stands can be classified into four types according to variations in canopy density and vegetation. This classification of forest stands was reasonable according to statistical analysis. The correlation coefficient between the variation rate of population density, Y_t, and major stand factors was larger than that of Y_t and the insect community indices. The results indicated that stand factors can be substituted for the effect of natural insect enemies of the pine caterpillar. Stand factors are easy to collect in the field and can be easily used in management models.

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Towards an Integrated Management of *Dendrolimus pini* L.

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ABSTRACT The pine moth *Dendrolimus pini* L. (*Lepidoptera: Lasiocampidae*) is one of the most serious forest insect defoliators in Poland. During the period 1946-95, more than 200,000 hectares of forests were treated in order to control this pest. In this paper, we present a historical background of pine moth outbreaks in Poland and review what is known about the biology of the insect and attempts to manage it. Important natural enemies and pathogens also are discussed. The idea of "the focus-complex method" and approaches to incorporate silvicultural methods into forest protection also are described.

OUTBREAKS OF THE pine moth *Dendrolimus pini* L. (*Lepidoptera: Lasiocampidae*) were first recognized in Poland at the beginning of the 17th century. The frequency of outbreaks appears to have increased over time (Szujecki 1993) (Table 1). During the period 1946-95, the pine moth was controlled in Poland on a total area of 233,000 hectares (Fig. 1), although there were 20 years during that period when no treatments were required.

Table 1. Pine moth outbreaks in Poland, 1791-1996

Years	Region of occurrence
1791 - 1792	West Pomerania
1863 - 1872	from Saxony to Masuria
1905 - 1909	Zagan Forest
1925 - 1927	Pomerania and Mazovia
1936 - 1937	Kurp Forest, Tuchola Coniferous Forest and Poznan province
1946 - 1952	West Pomerania, Zagan Forest, Kurp Forest and White Forest
1956 - 1957	Poznan and Tarnow provinces
1964 - 1975	from Notec Forest on the west to Augustow Forest on the north-east and Solska Forest
1982 - 1985	Pomerania and Zielona Gora province
1992 - 1996	Zielona Gora province, Tuchola Coniferous Forest, White Forest and Green Forest

The pine moth is a monophagous insect that feeds on Scots pine, *Pinus sylvestris* L. According to a 1996 report, Scots pine stands occupy approximately 69.4% of the forested area in Poland (Raport 1996). In the past, pure Scots pine stands were planted on sites that were both suitable and unsuitable for this species (Zasady 1994). The pine moth does not occur in stands that were planted on unsuitable sites which, according to Szujecki (1992), comprises 2.5 million hectares, or about 30% of the forested area in Poland. Long-term

Pages 129-142 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

observations indicate that young coniferous forests are most often infested by mass outbreaks of the pine moth. However, the pattern of pine moth outbreaks differs between western and eastern Poland (Lesniak 1976b).

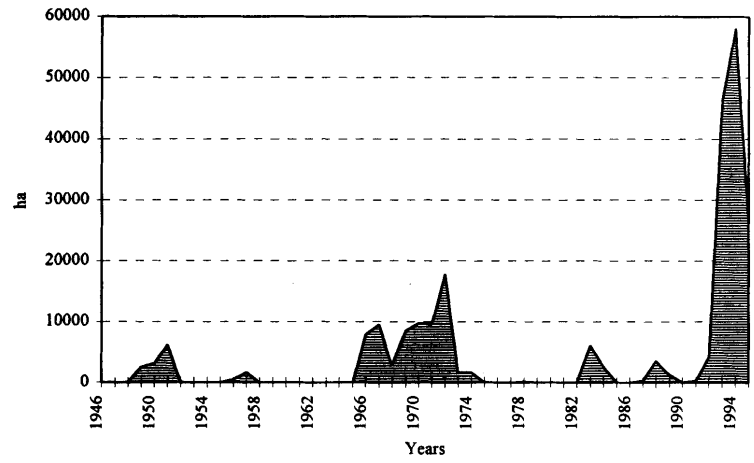

Figure 1. Control of pine moth in Poland, 1946-1996. Source of data: Department of Forest Protection, Forest Research Institute.

Lesniak (1976a) analyzed data from six meteorological stations between 1949 and 1970, and characterized abiotic conditions of the six identified centers of pine moth outbreaks. He suggested that climatic variation among different areas of Poland might significantly affect the course and intensity of outbreaks. He identified several climatic factors that were most important in population processes of the pine moth. These included: higher mean annual temperatures (particularly those during autumn and winter), lower wind velocities, less precipitation, fewer days with snow cover, the low mean long-term values for Seljaninov's coefficient[1], greater duration of summer and vegetation season, and higher frequency of dry months. Results of this research suggest that abiotic factors do not affect the pine moth directly, but rather the pine moth population dynamics indirectly, through the host plant.

Szujecki (1993) suggested that management systems in forestry (clear-cutting), positive thermal influences, and eutrophization of coniferous forest ecosystems, which deposit between 10 and 20 kg of nitrogen each year (Stachurski and Zimka 1984) may explain the increasing frequency of pine moth outbreaks. Contrary to studies by Luterek (1969) and Lesniak (1984), Szujecki (1993) showed that nitrogen fertilization positively influenced the development and fecundity of the pine moth.

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[1] Seljaninov's hydrothermal coefficient provides an example of a joint action of two fundamental abiotic factors. It is calculated according to the formula: (precipitation total x 10) divided by temperature (active) total. The coefficient values from 1.0 to 1.5 characterize an optimal humidity of a region, above 1.6 – its excess and below 1.0 – deficit. The most dangerous outbreaks of pine moth occur on areas in Poland with the lowest (1.2) value for Seljaninov's coefficient (Lesniak, 1976a).

Biology of Pine Moth in Poland

D. pini moths emerge from pupae in midsummer. Flight is observed from the beginning of July until the middle of August. Females usually do not fly before they mate, and they rest on the lower parts of pine tree trunks. Mating begins at night and lasts a few hours. Females can fly after having deposited most of their eggs, an activity that takes place in the early evening and at night. The moths live for 9-10 days. The females oviposit 150-250 eggs in groups of 20-100 on twigs without needles, on needles, or on the bark of Scots pine. It is very rare that females will deposit eggs on trees other than Scots pine.

Egg embryonation lasts 16-25 days. Larvae usually begin hatching in early September and are about 5 mm in length. When larvae complete their development, they are about 70-80 mm long and have increased their weight by about 700x. During the first 10 days, hatching larvae begin feeding on pine needles; at first, they feed only on the margin of the needles, and later, they consume entire needles. Before winter, larvae usually molt 2-3 times. Autumn feeding lasts until the first frost occurs (November). Then larvae move down the tree trunks to overwintering sites that are between the mineral soil and litter layer. About 85% of all larvae can be found in the immediate vicinity of tree trunks.

Early in the spring, usually in March, when soil temperature reach about 3°C, larvae begin to move upwards into the crowns. Depending on the weather condition, this activity can occur until the middle of April. In spring, the larvae consume 3-5 times more pine needles than in the autumn and they also can feed on the bark of young shoots. The studies carried out by Lesniak (1976c) confirmed that the spring-feeding larvae develop better on Scots pine needles from the previous year, whereas the autumn-feeding larvae develop best on the current year's needles. Before pupation, pine moth larvae are more active and can crawl for distances of several hundred meters to neighboring stands.

Pupation begins in May-June and lasts 4-5 weeks. Pupae are formed inside loose, partially transparent cocoons, and can be found in tree crowns, on the bark of trunks and on understory vegetation (Koehler 1961, Sliwa 1992).

Natural Enemies

The stabilizing influence of natural enemies in ecosystems has been reviewed by numerous authors (Sitowski 1928, Szmidt 1951, Szmidt 1955, Glowacka-Pilot 1974, Sliwa 1992) although there is lack of agreement about the relative importance of parasites, predators, and microorganisms in the natural control of the pine moth. The theoretical basis for the use of biological methods in forest protection were summarized briefly by Koehler (1968a).

Bacteria. The role of entomogenous bacteria and fungi in the regulation of pine moth populations in Poland was studied extensively by Glowacka-Pilot (1974). During 4 years, she isolated 374 strains of bacteria from 343 larvae (Table 2). Non-spore-forming species of bacteria were most responsible for disease in larval populations, and the most frequently isolated species was *Aerobacter cloaceae*. Although 29 strains belonged to the genus *Bacillus,* none of the isolates produced both spores and crystals.

Table 2. Bacteria isolated from pine moth larvae, 1965-69 (Glowacka-Pilot 1974)

Species	No. of isolated strains
1. *Achromobacter* sp.	14
2. *Aerobacter aerogenes*	33
3. *Aerobacter cloaceae*	101
4. *Bacillus brevis*	13
5. *Bacillus cereus*	7
6. *B. cereus* var *mycoides*	1
7. *Bacillus megaterium*	8
8. *Klebsiella aerogenes*	12
9. *Proteus rettgeri*	37
10. *Pseudomonas aeruginosa*	32
11. *Pseudomonas chlororaphis*	18
12. *Sarcina flava*	5
13. *Serratia marcescens*	93

In an earlier study (Glowacka-Pilot and Swiezyńska 1967), the insecticidal activity of two bacterial preparations, Thuricide (*B. thuringiensis* subsp. *thuringiensis*) and Dendrobacillin (*B. thuringiensis* subsp. *sotto* biotype *dendrolimus*), was compared. Results suggested that the insecticide based on *B. thuringiensis* subsp. *sotto* biotype *dendrolimus* might be more promising for the control of pine moth. Our unpublished data on the estimation of insecticidal activity of *B. thuringiensis* strains against pine moth larvae suggest that *B. thuringiensis* subsp. *sotto* biotype *dendrolimus* is more effective than *B. thuringiensis* subsp. *kurstaki* HD-1, which is used in most commercial preparations for forest protection.

Fungi. The role of pathogenic fungi in reducing the population density of the pine moth increases during the course of an outbreak. During a 4-year study Glowacka-Pilot (1974) isolated 1826 fungi strains from 1794 larvae (Table 3). The most frequently isolated species were *Paecilomyces farinosus* and *Beauveria bassiana*. *Verticillium lecanii* also was commonly found. *Cordyceps militaris* is another entomogenous fungal species that can be important in limiting pine moth populations. During the period of observation, this species occurred naturally in the several dozens of hectares in half of the outbreak areas and, in some places, it caused 30% to 80% mortality of overwintering larvae. Infection of pine moth larvae with the isolated fungi in laboratory conditions showed that *P. farinosus* and *B. bassiana* had the strongest pathogenic properties.

It is generally known that successful infection by fungi is related to relative humidity. The results of field trials on the infection of overwintering pine moth larvae by *B. bassiana* suggest that *B. bassiana* could be used as a natural regulator of pine moth population in those outbreak areas that are localized on coniferous forest sites with higher values of Seljaninov's coefficient. In the first trial, located in the central part of Poland, the mortality of overwintering larvae treated with *B. bassiana* was about 80% (Cichońska and Swiezyńska 1993). In the second trial, located in the western part of Poland on a dry coniferous forest site, the mortality of treated larvae was equal to the mortality of control larvae (unpublished data). This suggests that climatic conditions were responsible for the difference in the level of infection of *B. bassiana* against pine moth larvae.

Table 3. Fungi isolated from pine moth larvae, 1965-69 (Glowacka-Pilot 1974)

Species	No. of isolated strains
1. *Acremonium aranearum*	3
2. *Aspergillus parasiticus*	37
3. *Beauveria bassiana*	311
4. *Beauveria tenella*	7
5. *Verticillium falcatum*	10
6. *Verticillium lecanii*	179
7. *Verticillium* sp. I	1
8. *Verticillium* sp. II	1
9. *Cordyceps militaris*	542
10. *Fusarium* sp.	33
11. *Mucor* sp.	20
12. *Paecilomyces farinosus*	603
13. *Paecilomyces fumoso-roseus*	11
14. *Penicillium* sp.	30
15. *Scopulariopsis brevicaulis*	38

Parasites. The significance of insect species parasitizing the pine moth as natural regulators of pest population density differs depending on the stage of the outbreak and location. A listing of the more important pine moth parasites in Poland is provided in Table 4. For example, *Sarcophaga affinis* Fall. reduced pine moth larvae and pupae by 10% to 40%; similarly, *Exochilum giganteum* Grav. caused a reduction of 2% to 33% (Sitowski 1928). In 1947-48 and 1956-57, because of high parasitization of pine moth larvae by *Apanteles* sp. and *Meteorus* sp., the forest administration decided not to initiate control treatments (Sliwa 1992). In stands where sticky bands were used as a method of pine moth control, some activity by species of Muscidae was observed (Sitowski 1928). *Muscina pabulorum* Fall. parasitized 40-60% of pine moth larvae in the first year after applying sticky bands on tree trunks, and *Stomoxys calcitrans* L. parasitized up to 30% of the larvae.

Predators. The list of more important insect predators of pine moth is presented in Table 5. Birds also are important as pine moth predators, especially the cuckoo, golden oriole, starling, coal-tit, jay, thrush, rook, jackdaw, chaffinch and woodpecker. Among mammals, moles and bat are considered to be important (Koehler 1961).

Table 4. Parasites of nun moth (Sitowski 1928, Szmidt 1955, Koehler 1968a, Sliwa 1992)

Host stages	Order	Family	Parasite species
eggs	Hymenoptera	Proctotrupidae	*Teleas laeviusculus* Rtzb.
			Telenomus verticillatus Kiefer
			Trichogramma embryophagum Hart.
larvae	Diptera	Sarcophagidae	*Parasarcophaga harpax* Pand.
			Pseudosarcophaga monachae Kram.
			Sarcophaga affinis Fall.
			Sarcophaga tuberosa Pand.
		Tachinidae	*Tachina larvarum* L.
			Eriothrix rufomaculatus Deg.
			Sturmia scutellata R.D.
			Ernestia rudis Fall.
		Muscidae*	*Muscina pabulorum* Fall.
			Muscina stabulans Fall.
			Stomoxys calcitrans L.
			Amphiochaeta rufipes Meig.
	Hymenoptera	Ichneumonidae	*Exochilum giganteum* Grav.
			Pimpla inquisitor Scop.
			Pimpla instigator F.
			Pimpla holmgreni Schmiedekn.
			Theronia atalantae Poda.
			Pristomerus vulnerator Panz.
			Omorgus faunus Gr.
		Braconidae	*Apanteles ordinarius* Ratz.
			Meteorus versicolor Wesm.
		Chalcididae	*Tetrastichus xanthopus* Nees.
pupae	Hymenoptera	Chalcididae	*Tetrastichus xanthopus* Nees.

* Parasites found below sticky bands

Table 5. Predators of pine moth (Koehler 1961, 1968a, Sliwa 1992)

Host stages	Order	Family	Predator species
eggs	Raphidoptera	Raphididae	*Raphidia ophiopsis* L
larvae	Rhynchota	Pentatomidae	*Troilus luridus* L.
			Picromerus bidens L.
	Raphidoptera	Raphididae	*Raphidia ophiopsis* L
	Coleoptera	Carabidae	*Calasoma sycophanta* L.
			Carabus violaceus L.
			Carabus coriaceus L.
	Hymenoptera	Formicidae	*Formica polyctena* Forst.
			Formica rufa L.
pupae	Coleoptera	Carabidae	*Calasoma sycophanta* L.

Forecasting and Monitoring

Coniferous monocultures are especially susceptible to pest outbreaks and the application of sound forestry practices are required to assure their durability. According to Szujecki (1992), accurate forecasting of insect outbreaks and control of damaging insect populations are necessary to protect our forests against severe damage and economic losses.

Since 1946, yearly forecasts of the status of forest pest insect populations have been conducted in Poland. At present, it is based on the rules presented in "Forest Protection Guidelines" (1988). Data are collected on permanent plots in every State Forest District (SFD) (there are 443 SFD in Poland), and submitted, first to Regional State Forest Directorates (17), and then to the Forest Research Institute, Warsaw, where the actual forecast of the abundance of insect pests is developed.

Good knowledge of the biology of each pest allows us to formulate the most precise method for estimating the status of populations. If, for example, the number of larvae that collect below sticky bands that are placed on tree trunks of sampling trees exceeds the critical number for the stand (Table 6), aerial spraying is prescribed.

Table 6. Critical numbers of pine moth larvae (Burzynski 1988)

Stand age	No. of larvae per tree	Stand age	No. of larvae per tree	Stand age	No. of larvae per tree	Stand age
0 - 20	8 - 20	16 - 20	24 - 20	32 - 20	40 - 20	48 - 20
21 - 40	21 - 50	22 - 40	22 - 50	23 - 40	23 - 50	22 - 40
41 - 60	51 - 80	42 - 60	52 - 80	43 - 60	53 - 80	42 - 60
61 - 80	81 - 100	62 - 80	82 - 100	63 - 80	83 - 100	62 - 80
81 - 100	101 - 150	82 - 100	102 - 150	83 - 100	103 - 150	82 - 100

In the summer, observations of adult moth flight are performed at every SFD threatened with *D. pini*. The number of moths (females and males) that occupy trunks of control trees to a height of 4 m are counted. If mating is intensive, individual trees in the

threatened stands are cut after the larvae emerge and all larvae in the crowns are counted thoroughly. When the number of larvae found in crowns exceeds the critical number for the stand, aerial spraying can also be prescribed.

In order to optimize the forecasting of insect outbreaks, so-called "monitoring searching plots" were established in 1991. These plots were established to monitor populations in the stands where defoliation and discoloration of foliage has occurred. In 1991, there were 1,133 monitoring searching plots in pine stands in Poland (Ocena 1993). Methods of searching on monitoring plots have been developed so that the data obtained can be compared over time. At present, it is too early to reach conclusions about the effectiveness of these methods.

Past and Current Control of Pine Moth

Until the early 1960's, sticky bands were placed on tree-trunks in threatened stands as a means to control the pine moth. For example, in 1949 about 2,500 hectares of pine forest were protected against the pine moth using this method. Larvae that gathered below sticky bands attracted great numbers of parasites. Although the method was very effective, it also was very labor intensive. The first aerial spraying against the pine moth was conducted in 1951. Between 1951 and 1979, the insecticide DDT was used against the pine moth on a total area of 70,000 hectares (Sliwa 1992).

Table 7. Contact insecticides registered for ULV spraying in forestry in Poland (based on "Insecticides recommended for forest protection in 1996" ed. By Forest Research Institute, Warsaw)

			Recommended dose [l/ha]*	
Trade name	Common name	Active ingredient (%)	nun moth, *Acantholyda* sp., *Cephalcia* sp.	pine beauty moth, diprionids
Alfamor 05 SC	alphamethrin	5.0	0.15 - 0.20	0.12 - 0.15
Alfazot 05 EC	alphamethrin	5.0	0.15 - 0.20	0.12 - 0.15
Decis 2.5 EC	deltamethrin	2.5	0.25 - 0.32	0.15 - 0.25
Fastac 10 EC	alphamethrin	10.0	0.075 - 0.100	0.060 - 0.075
Karate 025 EC	lambda-cyhalothrin	2.5	0.25 - 0.32	0.15 - 0.25
Sumi-alpha 050 EC	esfenvalerate	5.0	0.20 - 0.25	0.15 - 0.20
Sumi-alpha 005 UL	esfenvalerate	0.5	2.00	2.00
Trebon 10 SC	etofenprox	10.0	0.40	0.30
Zorro 100 EC	zeta-cypermethrin	10.0	0.075 - 0.100	0.060 - 0.075

* Mixed with 0.70 l of mineral oil (IKAR 95 EC) and 1.50 - 3,00 l of water/hectare

In 1979, the use of DDT was prohibited in Poland in favor of synthetic pyrethroids. Pyrethroids are sill in use today (Table 7), together with insecticides that interfere with chitin deposition (Table 8), and commercial formulations of *Bacillus thuringiensis* (Table 9). Until the 1990's, diesel oil was often used as a carrier for chemical spraying; however, in 1994, we began to use an inflammable mineral oil as a carrier on large areas of sprayed forest (Biernacka et al. 1995). The two types of aircraft used most frequently for spraying the pine

moth are the PZL M-18 "Dromader," which has a maximum load of 2,500 kg and the Mi-2 helicopter, which has a maximum load of 400 kg. Since 1982, we have used ULV spraying to optimize control of pine moth populations.

Table 8. Chitin-inhibiting insecticides registered for forest managment in Poland (based on "Insecticides recommended for forest protection in 1996", ed. By Forest Research Institute, Warsaw)

Trade name	Common name	Active ingredient (%)	Recommended dose [kg or l/ha]	
			heavy infestation	normal infestation
Dimilin 480 SC	diflubenzuron	48	0.15	0.10
Dimilin 25 WP	diflubenzuron	25	0.30	0.25
Ekos 100 EC	heksaflumuron	10	0.50	0.40
Nomolt 150 SC	teflubenzuron	15	0.20	0.10

Table 9. *Bacillus thuringiensis* insecticides registered for forest management in Poland (based on "Insecticides recommended for forest protection in 1996", ed. By Forest Research Institute, Warsaw)

Trade name	B.t. strain	B.t. crystals (%)	Recommended dose [kg or l/ha]	
			heavy infestation	normal infestation
Bacilan(WP)	kurstaki HD-1	3.0	2.00	1.00
Bactospeine 16 000 WP	kurstaki HD-1	20.0	3.00	2.00
Dipel 8L	kurstaki HD-1	3.5	3.00	3.00
Dipel 3.2 WP	kurstaki HD-1	3.2	1.50	1.00
Ecotech Pro 07.5 OF	EG 2348	7.5	2.00	1.50
Ecotech Pro XL 015 OF	EG 2348	15.0	1.00	0.75
Foray 02.2 UL (48B)	kurstaki HD-1	2.2	4.00	2.50
Foray 03.3 UL (76B)	kurstaki HD-1	3.3	2.50	2.00
Thuridan-krem PA	kurstaki HD-1	3.0	2.00	1.50

There are two seasons during the year when pine moth can be treated effectively with insecticides: spring and autumn. There are advantages and disadvantages to spraying at these times. If the threatened area is well evaluated, spraying in autumn provides better foliage protection than spraying in the spring (Sliwa 1992). During the period 1946-1995, autumn spraying was conducted on about 10 occasions. Formulations of *B. thuringiensis,* toxicity of which is temperature-dependent, also can be applied in the autumn with satisfactory results (Fig. 2) (Sierpiñska and Sierpiñski 1994).

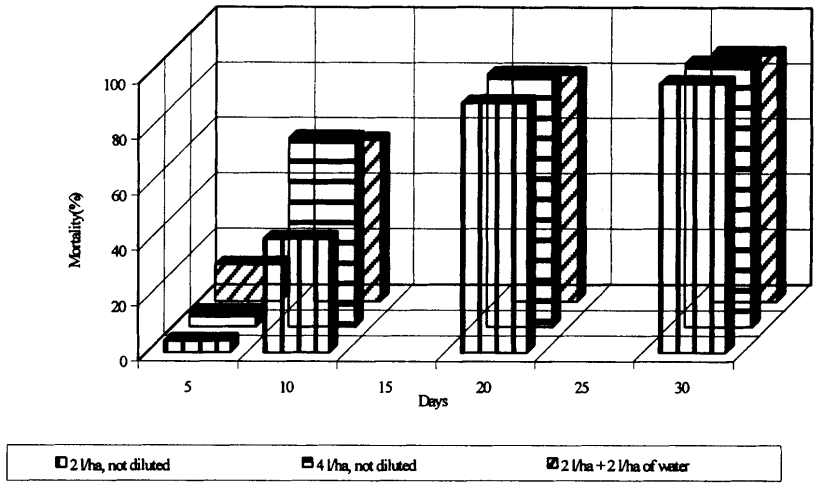

Figure 2. Mortality of pine moth larvae after treatment with Foray 48B, Autumn 1993 after Sierpinska and Sierpinski, 1994.

In August 1995, the efficacy of four ULV *B. thuringiensis* formulations and one pyrethroid was evaluated for the control of the pine moth. Aerial spraying was applied to 54 to 60 year old pine stands in which the mean number of larvae per tree was estimated to be 111 (21-161). An Mi-2 helicopter equipped with electric atomizers (AR 47.000) was used to apply the insecticide. Doses of insecticides and areas of sprayed plots are provided in Table 10. Before spraying, 1 m^2 linen cloths were placed under crowns of sample trees. The number of dead larvae on the linen cloths was checked on the 2nd, 7th, 10th, 15th, and 23rd day after treatment and calculated for the whole crown. On the last day, the sample trees were cut down, all dead and live larvae in the crowns were counted, and the percent mortality was calculated. After 23 days, the mortality of larvae sprayed with *Bt* insecticides was 97% and it was comparable to the mortality measured after treatment with pyrethroids (99%). The only difference was the time when the mortality at this level was observed. The larval mortality of 84% for the least effective *B. thuringiensis* product was observed after 23 days. Of the *B. thuringiensis* insecticides tested, Ecotech Pro XL provided the highest efficacy at the lowest volume (Fig. 3).

Table 10. Doses of insecticides (L/ha) used in 1995 experiments, and area sprayed

Insecticide	Dose [l/ha]	Area of sprayed plots [ha]
Dipel ESNT	2.8 (undil.)	101
Ecotech Pro 07.5 OF	2.0 (water dil.: 2.0 l/ha)	52
Ecotech Pro XL 015 OF	1.0 (water dil.: 1.5 l/ha)	407
Foray 76B	2.0 (undil.)	241
Zorro 100 EC	0.1 (+ 0.61 l IKAR 95 EC + 1.2 l water/ha)	97

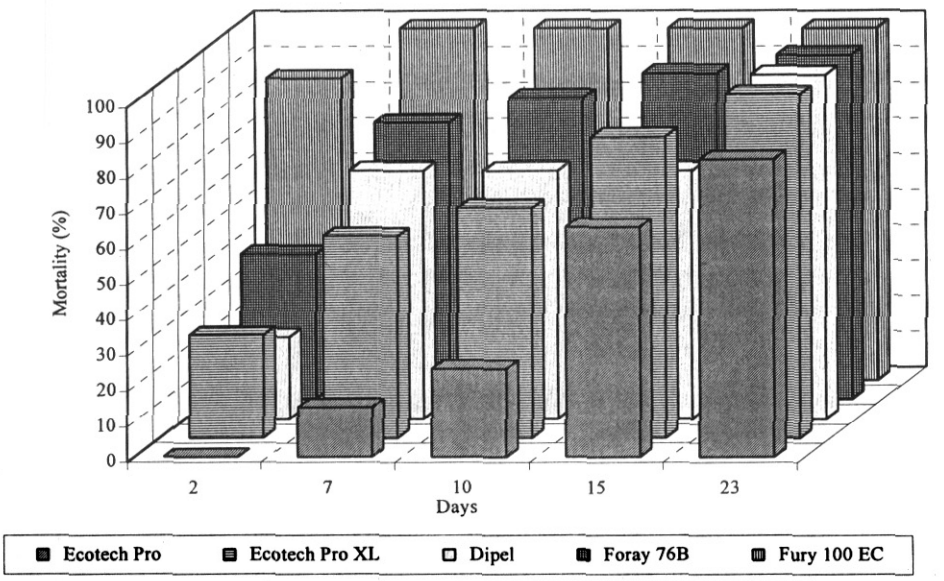

Figure 3. Mortality of pine moth larvae treated with four commercial formulations of *Bt* and a pyrethroid (Fury 100 EC).

Integration of Different Means and Methods

The first attempt at integrating different methods to protect forests and to apply prophylactic practices to improve the health of forests in Poland was referred to as the focal-complex (complex-center) method, which was formulated by Koehler (1968b).

The purpose of this approach was to initiate changes that develop gradually over time and lead to an equilibrium of less stable biocenoses in artificially regenerated stands. The "complex" depends on specific conditions of a definite forest area, e.g. diversity of the specific composition of the forest stand, protection of small mammals, increase in population density of bats and insectivorous birds, colonization of ants, concentration of parasitic insects, introduction of pathogens, etc. Intensive, integrated treatments were located in potential, primary centers of pest outbreaks.

The above described method applied not only to the pine moth situation, but resulted from an in-depth analysis of the causal sources of forest calamities and diseases in Poland.

One of the prescriptions of general importance in managed forest ecosystems to increase their resistance against insects attacks, is the utilization of silvicultural methods and the introduction of deciduous species to the understory of coniferous forests. Bernadzki et al. (1986) suggested that the introduction of lower forest layers favorably influenced the productivity of stands and the biocenotic potential of forest ecosystems, especially in more fertile habitats. They also suggest that this practice is justifiable in poor habitats because it reduces the economic risk, improves the quality of timber produced, influences the direction of succession processes, improves the state of the site, and also might create a microclimate that is unfavorable for insect pests and favorable for beneficial insects.

In 1994 the assumptions of the strategic government program designed to create a pro-ecological model of forestry were elaborated (Grzywacz 1994). The pro-ecological forest policy serves to integrate the protection of nature and forest management practices. The new, multi-functional model of forest management ensures the productivity, stability, and

usefulness of forest ecosystems for society (Rozwalka 1996). In agreement with the assumptions of the "Polish Policy of Integrated Protection of Forest Resources," Forest Promotion Complexes (FPC) have been established. At present, there are seven FPC in Poland which cover 300,000 hectares (5% of the forested area in Poland). The above mentioned assumptions of pro-ecological policy will become obligatory on state forest land in Poland; however, they will be introduced initially on the FPC areas (Szujecki 1996). The idea of establishing FPC seems to provide a good organizational framework for deploying Integrated pest management procedures and also for managing the pine moth.

Conclusions

Under existing conditions whereby Polish pine forest ecosystems are endangered by the repeated outbreaks of *D. pini*, the judicious use of insecticides is necessary in order to protect these forests from severe defoliation. During the past 50 years, a policy has existed whereby doses of chemicals used in forest protection should be the minimal dose required to provide effective control. During that period, insecticides like DDT, that were highly toxic to all elements of forest ecosystems, were withdrawn for control of pine moth and were replaced with more selective products such us *B. thuringiensis*-based preparations.

In Poland, *D. pini* has numerous natural enemies. Considering this, as well as the number and severity of forest protection problems in Poland, the creation of optimal conditions for naturally occurring pathogens, parasites and predators along with the use of silvicultural methods seems to be very important and can become an effective approach towards long-term sustainability.

Implementation of pro-ecological policy of "The Polish Policy for the Integrated Protection of Forest Resources" has created an organizational framework in which to implement integrated pest management.

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