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Recent Invasions of Five Species of Leafmining Lepidoptera in Hungary

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ABSTRACT Five species of leafmining Lepidoptera have expanded their range into Hungary during the last two decades. One of them (*Phyllonorycter leucographella*) is native to Southern Europe, while three species (*Argyresthia thuiella*, *Parectopa robiniella*, and *Phyllonorycter robiniella*) were accidentally introduced to Europe from North America. The origin of *Cameraria ohridella* is still not certain, although it was probably introduced from Asia. Three of the five species (*A. thuiella, Ph. Leucographella*, and *C. ohridella*) attack popular ornamental trees (*Aesculus, Pyracantha*, and *Thuja*) and the other two feed exclusively on black locust (*Robinia pseudoacacia*), an introduced, but widely planted, tree. The history of introduction, range expansion, and present and potential economic importance of these five insects in Hungary are discussed.

THE SPREAD OF organisms is always followed with great interest, particularly if they are exotic and have the ability to cause significant economic and ecological damage. In the last two decades, a number of insect species have expanded their ranges into Hungary. A number of exotic insects were accidentally introduced from North Africa, while others expanded their distribution from Southern Europe northward into Hungary. Many of these species have become established. These newly established insects include Lepidoptera, scale insects, gall wasps, and gall midges, and even dragonflies. Five species of leafmining microlepidoptera have invaded Hungary during the last 15 years. Three of them attack popular ornamental trees and shrubs (*Aesculus* sp., *Pyracantha coccinea* M.J. Roemer, and *Thuja* sp.), and the other two feed on black locust (*Robinia pseudoacacia* L.). Black locust was purposefully introduced into Hungary in the early 18th century and now comprises 20% of Hungarian forests.

The Invading Species

***Argyresthia thuiella* Packard 1871 (Argyresthiidae).** The wingspan of the adult of this leafmining moth is ca. 8 mm. The moths fly in early summer, and females lay their eggs on young leafelets. It has only one generation a year, unlike the other four leafmining species that have multiple generations. Larvae hatch within 1 to 2 weeks and the caterpillars mine the terminal shoots of the hosts, *Thuja occidentalis* (L.) (particularly the column-like form) and *Chamaecyparis lawsoniana* (A. Murr.) Parl. The larvae overwinter and later pupate in the mine. Symptoms of damage are browning and abscission of the attacked terminal shoots.

The species was accidentally introduced from Canada and the USA to Europe with *Thuja occidentalis* on three separate occasions: in 1971 to the Netherlands, in 1975 to Germany and in 1976 to Austria (Kurir 1983). From these introductions, it spread and was

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later recorded in several other European countries, including Switzerland (1989), Croatia (1991), and the Czech and Slovak Republic (1989) (Povolny 1990, Opalicki 1991).

The species probably spread into Hungary from neighboring Croatia. It was first recorded in the summer of 1997 near Zalaegerszeg in southwestern Hungary. It is possible that this insect was already present in Hungary prior to this, but population densities were below detectable levels. The distribution of this insect is currently restricted to southwestern Hungary, but will probably gradually spread and infest its hosts throughout the whole country. Because this species is univoltine and the host plants have an island-like distribution, a relatively slow rate of spread can be predicted. However, transporting infested ornamental plants within the country may help create newer foci of colonization and accelerate its spread within Hungary. The species has become part of the European fauna since its introduction in the 1970s and appears to have the ability to cause significant aesthetic and physiological damage to its hosts, two very popular ornamental plants.

***Phyllonorycter leucographella* (Zeller, 1850) (Gracillariidae).** The adult wingspan of this small leafmining moth is about 6 to 8 mm. The species usually has 2 to 3 generations per year in Hungary. Caterpillars feed in a long mine close to the upper leaf surface and overwinter in the mines. Its principle host is firethorn, *Pyracantha coccinea* M.J. Roemer, although occasionally it also feeds on *Crataegus* spp.

This insect is native to Southern Europe and was probably introduced accidentally into several countries in Western Europe on transported plant material. Firethorn is one of the most popular ornamental shrubs in many European countries. It keeps its leaves in the winter, and larvae overwintering in the mines are hard to detect. Because of this cryptic habit, it is very easy to unknowingly transport infected plant material.

In the 1970s, *Ph. leucographella* was found in France, Switzerland, and Austria. Since *Pyracantha* is native to the Mediterranean regions of France, it is possible it was always present there without being reported because of its cryptic habit. Even though this insect has not been reported from countries in Europe other than those mentioned above, it can be assumed that *Ph. leucographella* is likely to occur in Spain, Greece, Albania, Russia (Crimea), Turkey, and the southern part of the former Yugoslavia where its principle host plant is native.

This insect was later recorded in Germany (1980) and the Netherlands (1984), etc. (Stigter and Frankenhuyzen 1991, Sefrova 1999). By the mid 1990s, it had spread in continental Europe as far north as Denmark (Buhl et al. 1994). Considering the "jump-like" or multiple foci of spread within Europe, one may conclude that several separate introductions have occurred.

The first record of this insect in the United Kingdom was in 1989 (Emmet 1989). It is almost certain that the species was transported across the Channel on infected plants. Nash et al. (1995) studied the spread of this species in Great Britain. Besides the "natural" spread of the insect, they found several foci of colonization outside the main distribution range that were undoubtedly due to human activities.

This insect has spread eastward into Hungary and was first recorded in Sopron, near the Austrian border, in the fall of 1991 (Csóka 1992). The rate of spread within Hungary is slow compared to the spread of *Parectopa robiniella, Cameraria ohridella*, and *Phyllonorycter robiniella*. In nearly 10 years, it only advanced ca. 200 to 220 km eastward. The difference in the distribution of the hosts of these species may explain the difference in the rate of spread. *Aesculus* and *Robinia* can be found outside cities and villages as continuous rows of shade trees alongside roads, and *Robinia* is also grown in shelter belts. On the other hand, *Pyracantha* is restricted to cities and villages and is planted as an ornamental shrub, providing rather small and sporadic islands of the host plant, making colonization by this insect much more difficult.

***Cameraria ohridella* Deschka and Dimic, 1986 (Gracillariidae).** Adults of this species have a wingspan of ca. 6 to 8 mm. In Hungary it has 2 to 3 generations per year. The moths of the first generation fly in April and May. The first generation larvae mine leaves in the lower part of the crown. The 2nd and 3rd generations mine leaves higher in the crown, with the result that the leaves turn brown progressively from the bottom up through the crown. In heavy infestations, up to 100 to 150 mines can be found in a single leaf. The pupa overwinters in the leaf litter. Heavy infestations cause browning and early leaf fall in July, and repeated defoliation over several years can cause tree mortality.

Members of this family are genus monophagous on *Aesculus,* but can occasionally complete their development on *Acer platanoides* L. and *A. pseudoplatanus* L. (Gregor et al. 1998). The introduced North American species of *Aesculus (pavia*, *parviflora*, and *glabra*) appear to be more resistant than *A. hippocastanum*, a native of Europe.

The native distribution range of this species is not clear. The genus *Cameraria* was not represented by any other species earlier in Europe, but several species belonging to this genus can be found both in Asia and North America. This supports the idea that *C. ohridella* was introduced into Europe. The most likely hypothesis for its introduction into Europe is that the species was accidentally introduced from China into Albania in the last 30 years by immigrants and later spread on its own until it was first found at Lake Ohrid in Macedonia in 1985 and described as a new species a year later. Lake Ohrid is located very close to the Albanian border. It was not possible to check this hypothesis because until recently Albania was closed to outsiders and it is still closed to scientists from Europe. In the late 1980s the species expanded its range throughout the countries of the former Yugoslavia. In 1989 it was found in Zagreb, Croatia (Maceljski and Bertic 1995).

In 1989, *Cameraria ohridella* was discovered near Linz, Austria, approximately 1,000 km northwest of Lake Ohrid, Macedonia. It was introduced on purpose by an entomologist to study the species, unfortunately without considering the possible consequences of its escape. This Austrian location served as a starting point for invasion to many Central European countries. *Cameraria* spread from Linz in several directions, reaching Germany in 1992 (Heitland et al. 1999, Skuhravy 1999), the Czech Republic in 1993 (Skuhravy 1999), France in 1992 (Skuhravy 1999), Switzerland in 1998 (Kenis and Forster 1998), Slovakia in 1994 (Zúbrik 1998), and Poland in 1998 (Skuhravy 1999). Skuhravy (1999) summarized the expansion of the European distribution of this species. It is probable that the introduction of this insect into Hungary was from two different directions. Damage by this insect was first reported in 1993 in southwestern Hungary, very close to the Croatian and Slovenian border (Szabóky 1994), suggesting that this insect spread from the south into Hungary. In the following year (1994), very high population levels were reported near Sopron in northwestern Hungary, close to the Austrian border. It is highly likely the invasion near Sopron was from Austria, where the species was already common and abundant by that time. By 1997, the species had shown rapid expansion and was distributed throughout the whole country. The flying adults of the first generation probably crossed the Danube in 1994 where it flows from north to south in the middle of the country. The first reported signs of damage on trees east of the Danube were found in midsummer of 1994. At present, the insect can be found practically everywhere in Hungary where its hosts are present, and this insect is extremely abundant in many places. The host, *Aesculus* spp. (particularly *A. hippocastaneum*), is the most common and popular ornamental tree both in Hungary and Central Europe and can be found in nearly every town and village. Hickory is also one of the preferred shade trees along roads between towns and villages. These roadside trees probably accelerated the expansion of its range significantly. In addition, *A. hippocastaneum* is also planted in some forested areas in Hungary to increase the food supply for game animals, such as red deer and wild boars.

***Parectopa robiniella* Clemens 1859 (Gracillariidae).** This tiny leafmining moth has a wingspan of 5 mm and has 2 to 3 generations per year in Hungary. It is monophagous, feeding only on black locust, and its caterpillars make irregular, forking mines near the upper leaf surface. The caterpillars overwinter in cocoons spun on leaves and pupate in the spring. *P. robiniella* is native to North America and was accidentally introduced into Italy, where it was first found near Milan, northern Italy, in 1970 (Vidano 1970). From there it gradually spread in several directions. People probably facilitated this spread to several additional locations in Europe.

This insect was first reported in southern Hungary in 1983 (Maceljski and Igrc 1984). It spread relatively quickly within the country and within ca. 6 to 8 years became common everywhere in Hungary and very abundant in many places. It is interesting to note that in the first years of its invasion into Hungary, it was found mainly on small trees and sprouts of its host trees; it now also attacks mature host trees. Severe infestations cause early leaf fall as early as late June. Damaged trees often refoliate if weather conditions are favorable. The area damaged by this species has increased significantly during the last decade (Csóka 1998).

***Phyllonorycter robiniella* Clemens 1859 (Gracillariidae).** The adults of this small moth have a wingspan of 5.5 to 6.5 mm. Like *P. robiniellia*, this species is also monophagous on *Robinia pseudoacacia* and has 2 to 3 generations per year. The caterpillars make irregular, oval-shaped blotch mines on the underside of the leaves. Like the former species, it is also native to North America and is found there throughout the natural range of *Robinia pseudoacacia*. *Ph. robiniella* was accidentally introduced to Europe and first reported near Basel, Switzerland, in 1983 (Whitebread 1990). Later, it was also reported in France, Germany, northern Italy (1988), Austria (1989), and Slovakia (1992). It spread gradually through Austria, reaching Hungary in the mid 1990s. Damage (leaf mines) caused by this insect was first observed in the autumn of 1996 in northwestern Hungary (Szabóky and Csóka 1997). However, according to Sefrova (pers. comm.), it was already present in Hungary in 1993. It causes the same type of damage as *Parectopa robiniella* – leaf fall in early or mid summer. These two species often occur together. The long-term impact of repeated defoliation by this insect is as yet unkown.

The rate of spread of this species increased significantly after reaching the Hungarian border. In 2 years it had practically invaded the whole country, a distance of ca. 500km from west to east. The extremely fast spread of these two species (*Ph. robiniella* and *Parectopa robiniella*) on black locust, particularly *Ph. robiniella*, is a phenomenon unique to Hungary because black locust, the most abundant tree species, is found throughout the whole country. Black locust stands comprise more than 340,000 hectares, or 20% of the forested area in Hungary. This tree was introduced in the early 18th century and later was widely planted throughout the country. Hungary currently has more black locust stands than all other European countries combined. Widespread planting programs were justified by the desirable characteristics of this species: it grows fast (even on relatively poor sites), the timber is hard and quite resistant to woodrot, and its wood is also excellent for firewood. The tree is also good for honey production and does not have any serious pests. The last reason may no longer be accurate and justified. Because of the importance of this host plant in Hungary, the last two species of leafminer have the potential to cause negative economic impacts in general and have very serious effects on the Hungarian forest industry in particular, probably greater than anywhere else in Europe.

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Implementation of a Program to Optimize the Use of *Bacillus thuringiensis* Against the Browntail Moth (*Euproctis chrysorrhoea)*

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ABSTRACT Laboratory and field studies were conducted over a period of 3 years to develop formulations of *Bacillus thuringiensis* (*Bt*) that would be efficacious against the browntail moth (*Euproctis chrysorrhoea)*, a serious pest of forest and shade trees and shrub vegetation in coastal areas of Maine and Massachusetts. Standard feeding bioassays and a novel electrophysiological procedure were used to determine the susceptibility of browntail moth larvae to the insecticidal crystal protein toxins that are incorporated into commercial formulations of *Bt.* The electrophysiological method, voltage/current clamp analysis, is discussed in detail.

THE BROWNTAIL MOTH (*Euproctis chrysorrhoea)* (BTM), is found throughout Europe, northern Africa, and Asia, where it defoliates orchard, forest, and shade trees (Lipa 1996). In the United Kingdom it is found most frequently on woody Rosaceae and on fruit trees in urban and suburban areas (Kelly et al. 1989). In Croatia, *E. chrysorrhoea* is a serious pest in English oak (*Quercus robur)* forests, 6,000 to 10,000 ha of which were sprayed annually with a pyrethroid insecticide during a recent outbreak (B. Hrasovec, pers. comm.). Each year, BTM larvae damage cork oak (*Q. suber)* plantations in southern Europe and stands of deciduous trees in central and eastern Europe. Outbreaks occur at intervals of 7 to 8 years and last 3 to 4 years (Lipa 1996*)*. In addition, both larvae and pupae are covered with urticating hairs that can cause a severe and persistent skin rash on humans and trigger an asthmatic reaction in sensitive individuals (Blair 1979).

E. chrysorrhoea was accidentally introduced into the United States in 1897 near Somerville, Massachusetts (Schaefer 1989). By 1914, the BTM was found throughout most of New England and southern Canada. Populations declined thereafter and by 1960 there were residual populations only on the outer dunes of the Cape Cod National Seashore and on the islands in and around Casco Bay, Maine.

However, there was a subsequent resurgence of *E. chrysorrhoea* populations that began in 1989. By 1995, species of *Quercus,* serviceberry (*Amelanchier canadensis)*, beach plum (*Prunus maritima)*, and bayberry (*Myrica pensylvania)* were severely defoliated on 28 islands in Casco Bay, while the infestation on the mainland encompassed 176,000 ha. On the National Seashore, the Park Service is concerned about the impact of *E. chrysorrhoea* on the shrub vegetation (Rosaceae) that stabilizes the outer dunes, the effect of urticating hairs on the high-density visitor population, and the displacement of native species. In the Casco Bay area, concern is focused on defoliation, unsightly webs, and public health on the inhabited islands and heavily populated coastal areas. Beginning in the mid 1980s, attempts were made to control the BTM on shrub vegetation on National Park Service lands by mechanically

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clipping overwintering webs and destroying them. However, this approach is not practical where webs are on terminal branches of oak trees that are inaccessible from the ground. Although a chemical pesticide, diflubenzuron, is effective against the BTM, it has a long residual and is toxic to certain aquatic arthropods. Residents in and around Casco Bay expressed concern about the proximity of spray blocks to the ocean and possible adverse effects of diflubenzuron on the shellfish industry and the commercially important American lobster (*Homaris americanus*).

Early attempts to control BTM populations with commercial formulations of *Bacillus thuringiensis* (*Bt*), a microbial pesticide that is used successfully against the closely related gypsy moth (*Lymantria dispar* L.) and other Lepidoptera pests, were not effective (Bradbury 1995). A series of studies was initiated in 1995 to determine the reason for the poor performance of *Bt* against the BTM and to develop and evaluate novel formulations of *Bt* that could be used to control BTM populations in environmentally sensitive habitats.

Materials and Methods

Rearing Methodology. Laboratory bioassay studies on BTM larvae were initiated in June 1995. Late-stage larvae were collected from an infestation in Maine and returned to the Northeastern Research Station laboratory at Hamden, Connecticut. Repeated attempts to obtain data using standard larval bioassay procedures with both oak foliage and artificial diet (Bell et al. 1981) were unsuccessful because larvae would not feed on the diet and consumed little of the oak foliage.

In March 1996, we began working with second-stage larvae that emerged from overwintering nests to establish a lab rearing protocol. Larvae that were offered potted oak (*Q. rubra)* seedlings initially skeletonized the leaves but did not survive to the third stage. Attempts to transfer larvae to fresh foliage resulted in cessation of feeding by the entire colony. It became apparent that any disruption in the integrity of the larval colony and associated webbing, e.g., to thin the number of larvae to a manageable number (10 to 20) for bioassay purposes, caused the larvae to discontinue feeding.

We then attempted to establish colonies in the laboratory from third and fourth instar larvae that had been collected in the field along with their communal webs that were interwoven with leaves and twigs. Larvae would not feed when transferred to potted oak seedlings but fed on freshly cut oak foliage if the entire colony was placed near the foliage bouquet. Using this technique, we were able to initiate bioassays by treating foliage bouquets with a predetermined dose of *Bt* using a spray tower (Hubbard and Lewis 1973) and then placing the treated bouquets in a small plastic cage along with the entire colony. This procedure was not wholly satisfactory because we could not control the number of larvae per replicate (colony) before treatment, nor determine the actual number of larvae/replicate until the end of the bioassay period when we opened the nests and separated the larvae. Although the response of the larvae to *Bt* using this procedure was extremely variable, we were able to obtain important initial information.

Efforts were then directed at establishing BTM larvae on an artificial diet (Bell et al. 1981). We collected egg masses from the field in 1996, placed the entire egg mass directly on the artificial diet, and incubated egg masses under several temperatures and light regimes. We determined that emerging BTM larvae will adapt to artificial diet, forego diapause, and develop to adults under the following conditions: (1) place the egg mass in a 6-oz Dixie Cup half filled with Bell diet and incubate under continuous light at 22 to 24°C and 30 to 40% RH; (2) after eclosion, allow the larvae to form a heavy web on the surface of the diet for 7 to 10 days; (3) transfer the larvae and webbing gently to fresh diet and incubate for 10 days; and (4) at this point, thin the colony to ca. 50 larvae per cup and use in feeding bioassays.

Electrophysiological Studies. Preliminary feeding bioassays suggested that BTM larvae were not highly susceptible to commercial *Bt* formulations used commonly against the gypsy moth and other forest Lepidoptera. Since there were no data available on the susceptibility of BTM to different insecticidal crystal proteins (ICP) that are present in commercial formulations of *Bt* and because of the difficulty encountered in developing a standardized bioassay procedure such as that which exists for the gypsy moth (Dubois 1986) and other species, we recognized the need for a more efficient procedure.

With assistance from Prof. Donald Dean, Ohio State University, we began electrophysiological (voltage clamp) bioassays of specific purified ICPs against excised midguts of the BTM, using the procedure described by Liegig et al. (1995), to determine their binding characteristics. Previous studies have shown that the site of action of the Cry classes of ICP toxins is the membrane of the midgut cells, and that the binding characteristics of specific ICPs to midgut cell receptors correlate closely with their toxicity to individual species.

Fourth-stage larvae collected from the Casco Bay area were used in the initial studies of purified ICPs. *Bt* formulations containing the same toxins were used to challenge BTM larvae at the equivalent dose of 10 BIU/ha. Four replicates of 20 larvae each were assayed and treatment effects were analyzed by ANOVA; means were compared using an unpaired t test.

Ground Application. Laboratory bioassays conducted in 1996 indicated that the BTM is most sensitive to the CryIAc toxin that is incorporated as one of the complex of toxins found in most commercial formulations of *Bt*. The objective of the 1997 ground application study was to evaluate the efficacy of CrIAc alone and CryIAc added to Condor OF® and Foray 48B® against the BTM when applied at the highest registered dose of 98.8 BIU/ha using a conventional mist blower. CryIAc was provided by Mycogen Corporation as a pure toxin preparation under the name MVPII®.

Three 0.4-ha blocks separated by a 16.7-m buffer zone were established for each treatment and the untreated control. Six sleeve cages were placed at random on northern red oak (*Quercus rubra)* branches in each block immediately after spray application; a minimum of 50 third and fourth instar larvae were placed in each cage. Counts of live and dead larvae were made at 4, 8, 12, and 16 days after spray and dead larvae were removed after being counted. Additionally, six branches containing BTM larvae were placed in plastic bags, chilled, and returned to the lab where they were placed in Melrose boxes. Mortality of larvae was recorded daily for 10 days.

MVPII® was applied at a rate of 954 nanograms of active ingredient (AI) per cm^2 of leaf surface. Condor OF® and Foray 48B® were applied at 98.8 BIU/ha with the same concentration of MVPII® added to the mixture.

Aerial Application. Based on the encouraging results of the ground application conducted in 1997 and additional bioassays that were conducted over the winter months, an aerial spray trial was conducted in May 1998 to determine the efficacy of Foray 48B® and MVPII® applied at 39 oz/ha at a ratio of 0.6 to 1 against BTM larvae. The test was conducted on Peaks Island, located within the City of Portland, Maine. This area was chosen because it supported a light to moderate (3 to 10 overwintering webs/northern red oak) population of the BTM.

An 81-ha block was established and marked for treatment. Twelve sleeve cages were distributed at random throughout the block and attached to northern red oak branches; 20 larvae were placed within the cages within several hours after the *Bt* formulation was applied. An equal number of cages were established in the same way in unsprayed areas on the island where larval densities based on the number of webs/tree were similar to those in the treated area. Counts of live and dead larvae within the cages were made immediately after treatment and 4, 8, 12, and 16 days after spraying. Dead larvae were removed after each observation.

The *Bt* formulation that was tested consisted of two parts of Foray 48B® to three parts of MVPII® applied undiluted at 7.0 l/ha. In terms of active ingredients, this dose represented 35.3 BIU/ha of Foray 48B® and 117.999 g/ha of MVPII®. A Thrush Commander aircraft equipped with 8-AU 5000 Micronair nozzles was calibrated to deliver droplets within a range of 125 to 135 μ VMD (volume mean density). Treatment commenced on the morning of May 18, 1998.

Results

Laboratory Bioassays. Foliage bioassays with commercial formulations of *Bt* with known ICP composition provided the first clue that the BTM was most susceptible to the CryIAc toxin (Table 1).

Table 1. Simulated aerial application of *Bacillus thuringiensis* formulations at 8 BIU/acre equivalent against fourth and fifth instar browntail moth larvae (mortality after 5 days)

Formulation	ICP Composition (Cry)							Dead	C.V.[a]
	IAa	IAb	IAc	IB	IC	ID	IIA		
Control								6.7	1.71
Foray 48B®[b]	x	x	x					86.2	0.08
Dipel 6AF®	x	x	x					58.3	0.84
Condor OF®	x		x				x	76.9	0.49
Xentari®	x	x						22.1	1.40
CryMax®	x				x		x	38.8	1.06

[a] Four replicates with 50 to 250 larvae per replicate

[b] Dose used was 4 BIU/acre equivalent

Pure toxin feeding bioassays on foliage bouquets confirmed that CryIAc without spores caused significantly higher mortality than CryIAa or CryIAb (Table 2).

Table 2. Pure insecticidal crystal protein (ICP) bioassays with and without HD-73 spores against fourth and fifth instar browntail moth larvae

Preparation	Spores	Percent Mortality at Day 6	
		Actual	Corrected[b]
Foray 48B®[a]	+	46	31
CryIAa	-	46	31
CryIAa	+	41	24
CryIAb	-	41	24
CryIAb	+	17	0
CryIAc	-	82	77
CryIAc	+	88	85
Controls	0	22	

[a] = 8 BIU/acre equivalent CP provided by Mycogen Corp. as MVPII® (pseudomonas encapsulated) formulations and applied on bouquets at 3.6 to 4.0 grams/ml/bouquet. Spore concentration = 25 per application (3 reps, 250 larvae/rep)

[b] Corrected by Abbott formula

Augmentation of Foray 48B® or Condor OF® with MVPII®, a commercial preparation of CryIAc, resulted in significant increase in mortality compared to that with either *Bt* formulation alone. A low mixture ratio of *Bt*:MVPII® (0.6:1) increased the efficacy of the Foray 48B® formulation significantly; a ratio of 5.3:1 increased the efficacy of Condor OF®.

Electrophysiological Studies. Voltage clamp assays conducted on the isolated midguts of fourth and fifth instar larvae confirmed that, based on the inhibition of electrical activity (-μA/minute), the BTM is much more susceptible to the CryIAc ICP (Table 3).

Table 3. Rate of short-circuit current inhibition (-μA/minute) of browntail moth larval midguts by activated insecticidal crystal proteins (ICP) of *Bacillus thuringiensis*

ICP	-μA/minute	Mean	R^2	% μA loss/minute	Mean
CryIAa	-0.182		72	1.82	
	-0.187		96	2.67	
		-0.185			2.25
CryIAb	-0.346		95	2.31	
	-0.121		94	1.34	
		-0.234			1.83
CryIAc	-0.283		98	3.14	
	-0.401		98	3.48	
		-0.342			3.31
CryIIA	-0.065		89	1.63	
	-0.033		72	1.63	
		-0.049			1.63

Ground Application. In the field study, the 5.3:1 mixture of *Bt*: MVPII® resulted in significant larval mortality in the Condor OF® plot 4 days after treatment and 3 days later in the Foray 48B® plot (Table 4).

Table 4. Percent mortality of browntail moth larvae in sleeve cages after treatment with MVPII®, Condor OF® + MVPII® or Foray 48B® + MVPII®, May 29, 1997

	Treatment				ANOVA		
Date	Control	MVPII®	Condor OF® + MVPII®	Foray 48B® + MVPII®	df	F	P
6-03	4.0ac[a]	1.4	13.6B	7.8AB	3,68	9.988	0.000
6-06	4.7a	5.0a	24.7b	17.1b	3,68	15.330	0.000
6-10	5.2a	12.0	41.0b	32.2b	3,68	28.437	0.000
6-13	6.3a	30.7b	71.2b	77.7b	3,68	75.034	0.000
6-19	47.1a	79.6b	98.2b	98.8b	3,68	31.805	0.000

[a] Numbers in rows with the same letter are not significantly different from each other (Tukey HSD Multiple Comparison at $P < 0.05$)

However, there was no difference in the cumulative mortality of larvae between the two *Bt* formulations. MVPII® alone did not differ significantly from the controls until 2 weeks after treatment.

Aerial Application. Mortality of the caged larvae 21 days after treatment was 72% in the treated plot and 16% in the controls (Fig. 1). We attributed the abnormal mortality in two of the control cages to the fact that they had been inadvertently placed near an area that had been sprayed with diflubenzuron. The mean percent mortality in the other 10 sleeve cages was 10.2%.

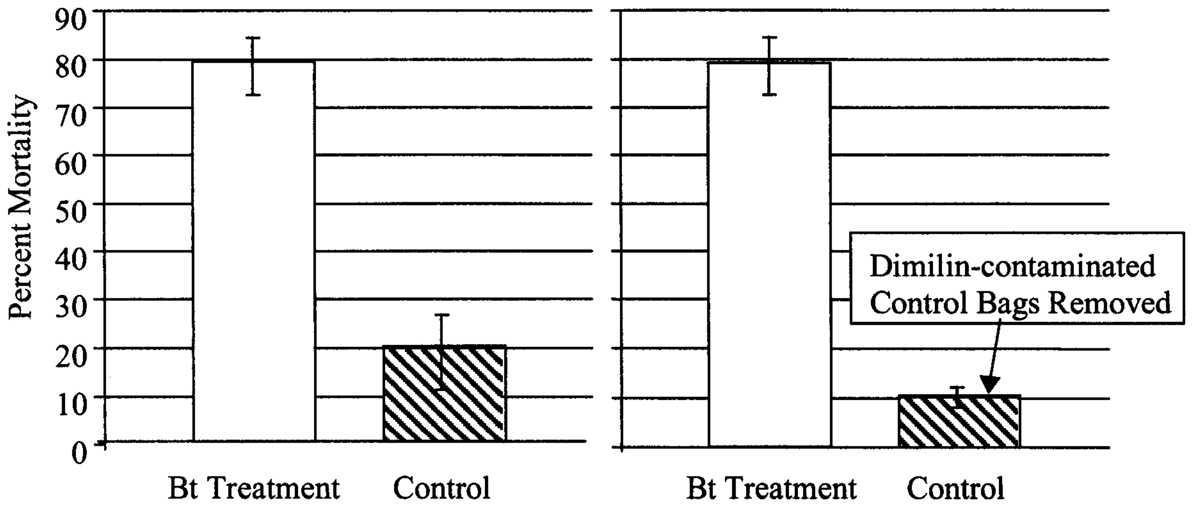

Figure 1. Results of aerial spray trial conducted on Peaks Island, Maine, May 18, 1998. Foray 48B® and MVPII® were applied at 39 oz/ha, a ratio of 0.6 to 1.0.

Discussion

That the BTM is well adapted to survival in rigorous environments makes it difficult to control this species. Communal webs provide a means for all siblings from a single egg mass to overwinter (Schaefer 1989). After completing diapause, larvae feed gregariously in the spring and summer months and produce large communal webs, some of which are more than 30 cm long (Bradbury 1995). The colonial feeding behavior of BTM larvae complicated our attempts to conduct routine bioassays in the laboratory to determine their susceptibility to commercial formations of *Bt* and its ICPs. Although the results of our efforts to overcome this behavior were not entirely satisfactory, we obtained preliminary information suggesting that the response of BTM to commercial *Bt* formulations differed substantially from that of other forest defoliators, e.g., the closely related gypsy moth. These findings provided the stimulus to explore the use of an electrophysiological method as an alternative to standard feeding bioassays that have been used successfully in the past (Dubois 1986). The following section is a brief review of the *Bt* endotoxins (ICPs) and their relationship to voltage/current bioassay procedures

Strains of *Bt* are pathogenic to species within the Lepidoptera, Diptera or Coleoptera depending on the type of delta endotoxins (ICPs) that they produce. The CryIA class is specific to Lepidoptera, the CryII class to Lepidoptera and Diptera, the Cry III class to Coleoptera, and the Cry IV class to Diptera (Dubois et al. 1997). These toxins are selectively toxic to cells in the midgut epithelium of susceptible species.

After they are ingested, the ICPs are solubilized by high ph (>10) in the midgut of larvae to a protease-resistant toxin that binds to specific receptor sites on the brush border membrane of the columnar cells. It has been suggested that this toxin disrupts the transport of potassium (K+), which results in physiological changes in the function of the columnar cells. These cells absorb water, swell, and lyse, resulting in partial destruction of the midgut. This process can be measured by increased short-circuit inhibition across the midgut membrane (Harvey and Wolfersberger 1979). The authors found that 60% of the short-circuit current was inhibited when *Bt* was administered to the isolated midgut of *Manduca sexta*; electrical resistance was reduced by 55% and oxygen uptake was stimulated by ca. 30%. This sequence of events occurs 30 to 40 minutes after ingestion of *Bt* by susceptible larvae and is followed by death from starvation or septicemia 48 to 72 hours later. The voltage/current clamp procedure and the chamber that is used to measure these changes are described in Wood and Moreton (1978).

The voltage/current bioassay procedure was effective in evaluating the susceptibility of BTM larvae to the ICPs used in commercial formulations of *Bt.* This procedure can be used as an alternative to costly and time-consuming feeding bioassays. The results of this procedure, indicating that BTM larvae were more susceptible to the CryIAc insecticidal crystal protein of *Bt*, were confirmed in truck-mounted mistblower applications in 1997 and in aerial applications in 1998. Consequently, a modified commercial formulation of *Bt* that is efficacious against the BTM was developed and evaluated for 3 years. This formulation is a safe alternative to registered chemical contact pesticides and is especially effective in managing BTM populations on environmentally sensitive lands where the use of more efficacious products may adversely affect nontarget species and pose a health risk to humans.

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Implications of Non-Indigenous Insect Introductions in Forest Ecosystems

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ABSTRACT The status of non-indigenous introductions as forest pests in Canada and ongoing research on species associated with solid wood packaging are briefly reviewed. Introductions began soon after the start of European colonization, with many of the early arrivals being of little consequence as pests. Since then, however, a significant number of serious forest insect pests and diseases have reached North America. Some have forever altered forest ecosystems. Through containment rearing of solid wood packaging intercepted in the port of Vancouver, British Columbia, we demonstrate that serious forest pests continue to arrive in association with a variety of commodities. In research studies in urban reserves and forests adjacent to the port, we have found evidence that non-indigenous species of ambrosia beetles and woodborers now predominate in some communities. The potential effects of these species on forest ecosystems are unknown. Adoption and enforcement of regulations to ensure that only pest-free solid wood packaging is used in international trade are required before introductions of invasive bark and woodborers will cease.

THE PURPOSE OF this presentation is to acquaint or reacquaint you with the breadth and magnitude of problems arising from non-indigenous species introductions and establishments, and to inform you of some of the efforts currently underway in Canada to address the issue.

Non-indigenous insects are a threat to forest ecosystems and forest economies. There is ample evidence from around the world of the devastating effects resulting from the intentional or inadvertent movement by humans of plant and animal species outside of their natural range. The majority of the introduced species in North America originated from Europe or Asia as a result of trade with those continents over the past several hundred years. Most of the historical introductions were inadvertent, although on occasion they have been the result of well intentioned but poorly thought out species transfers (e.g., gypsy moth).

Historical Establishments

The first adventive non-indigenous species likely arrived soon after the discovery and colonization of the continent by European explorers and settlers. Some of the first arrivals were ground beetles inadvertently transported with stone and gravel used as ships ballast. Disposal of the ballast into the sea was prohibited by decree to maintain the quality of anchorages in early fishing ports. The ballast, contaminated with the plant and insects of coastal Europe, was deposited on shore (Turnbull 1979). The legacy of this practice is evident today in the disjunct distributions of Carabidae along the east and west coasts of the continent and waterways of the Saint Lawrence River and Great Lakes and in the high proportion of European species present in the fauna of Newfoundland (>13%), the region

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Pages 45-55 in Liebhold, A.M.; McManus, M.L.; Otvos, I.S.; Fosbroke, S.L.C., eds. 2001. **Proceedings: integrated management and dynamics of forest defoliating insects**; 1999 August 15-19; Victoria, BC. Gen. Tech. Rep. NE-277. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station.

with the earliest and hence the longest period of settlement in Canada (Scudder 1979, Bousquet 1991).

While many of the earliest arrivals had little impact on forest ecosystems in Canada, further colonization and trade, primarily with Europe, eventually led to the establishment of an increasing number of forest pests in our forests. Some of the more significant non-indigenous insects and diseases established in Canada's forests, the dates of their first discovery, and the tree species attacked are noted in Tables 1 and 2. Liebhold et al. (1995) provide case histories for and review the impacts of a range of non-indigenous forest pests introduced into North America, including the gypsy moth, pine shoot beetle, chestnut blight, and white pine blister rust. These and other introduced taxa have modified forest ecosystems (e.g., virtual elimination of American Chestnut from eastern forests); affected the timber, recreational, and wildlife uses of forest resources; and threatened the viability of endangered species or ecosystems.

Table 1. Significant forest insect pests introduced into Canada (after Anon. 1999)

Insect	Year Introduced	Primary Hosts
*Larch sawfly (*Pristiphora erichsonii*)	1882	larches
Browntail moth (*Euproctis chrysorrhea*)	1902	all deciduous species
Poplar sawfly (*Trichiocampus viminalis*)	1904	trembling aspen, largetooth aspen, balsam poplar
*Larch casebearer (*Coleophora laricella*)	1905	larches
Late birch leaf edgeminer (*Heterarthus nemoratus*)	1905	birches
*Balsam woolly adelgid (*Adelges piceae*)	1908	balsam fir, grand fir, subalpine fir, Pacific silver fir
*Satin moth (*Leucoma salicis*)	1920	poplars
*European spruce sawfly (*Glipinia hercyniae*)	1922	spruces
*Gypsy moth (*Lymantria dispar*)	1924	oaks, birches, larches, willows, basswood, Manitoba maple
*European pine shoot moth (*Rhyacionia buoliana*)	1925	red pine, jack pine, Scots pine
*Winter moth (*Operophtera brumata*)	1920s	oaks, maples, willows
*Mountain-ash sawfly (*Pristiphora geniculata*)	1926	mountain-ash
*Birch leafminer (*Fenusa pusilla*)	1929	birches
Introduced pine sawfly (*Diprion similis*)	1931	pines
Birch casebearer (*Coleophora serratella*)	1933	poplars
*European pine sawfly (*Neodiprion sertifer*)	1939	red pine, Scots pine
Elm leaf beetle (*Pyrrhalta luteola*)	1945	elms
Smaller European elm bark beetle (*Scolytus multistriatus*)	1946	elms
Ambermarked birch leafminer (*Profenusa thomsoni*)	1948	birches
*Apple ermine moth (*Yponomeuta malinella*)	1957	apple
European pine needle midge (*Contarinia baeri*)	1964	red pine, Scots pine
Early birch leaf edgeminer (*Messa nana*)	1967	birches
*Pine false webworm (*Acantholyda erythrocephala*)	1961	pines
Pear thrips (*Taeniothrips inconsequens*)	1989	sugar maple, red maple
Pine shoot beetle (*Tomicus piniperda*)	1993	pines, spruces

* Denotes species for which biological control programs have been implemented

Table 2. Significant forest diseases introduced into Canada (after Anon. 1999)

Disease	Year Introduced	Primary Hosts
Dothichiza canker (*Cryptodiaporthe populea*)	pre 1900	Poplars
Chestnut blight (*Cryphonectria parasitica*)	post 1904	American chestnut
White pine blister rust (*Cronartium ribicola*)	1910	White pine
Willow blight (*Venturia saliciperda*)	ca. 1925	Willows
Dutch elm disease (*Ophistoma ulmi*)	1944	Elms
Scleroderris canker (European race) (*Gremmeniella abietina*)	1978	Pines
European larch canker (*Lachnellula willkommii*)	1980	Larches
Beech bark disease (*Nectria coccinea var. faginata*) and beech scale (*Cryptococcus fagisuga*)	1980	American beech
Butternut canker (*Sirococcus clavigignenti*)	1991	Butternut

While in Victoria, you will have the chance to visit one of the most threatened ecosystems in Canada, the communities dominated by Garry oak (*Quercus garryana* Dougl.) on southeastern Vancouver Island and the adjacent Gulf Islands. This community has been impacted by serious levels of defoliation over the past three decades caused by a sequence of exotic introductions. Much of the original Garry oak woodland has been lost to urbanization. The remaining stands are threatened by urban expansion as well as the effects of defoliation resulting from non-indigenous introductions. More than 800 species of arthropods are associated with Garry oak in this area (Evans 1985). At least 140 of these species are herbivores, feeding on the foliage, acorns, branches, trunk or roots of the tree. One third of the herbivores (48 spp.) are restricted to Garry oak alone. At least 10% of the herbivores (14 spp.) now present on Garry oak are non-indigenous introductions (Table 3). Three of the introduced species (winter moth, the oak leaf phylloxeran, and jumping gall wasp) have adversely affected the health and survival of Garry oak through repeated defoliation or scorching during prolonged outbreaks (Van Sickle 1995; Duncan 1997a,b). The establishment of another exotic oak pest, the gypsy moth, has so far been prevented through ongoing detection and eradication programs (Humble and Stewart 1994). This year, more than 12,000 ha of southern Vancouver Island, including significant areas containing Garry oak, have been treated to eradicate a recently introduced population of gypsy moth.

Current Research

As a consequence of the serious impacts historical non-indigenous introductions have had on agricultural and forest economies, inspection and quarantine systems have been implemented by most nations to prevent introductions of new harmful invasive species or to limit the spread of already established species. In spite of these regulatory efforts, additional non-indigenous species are discovered annually. Factors contributing to the continued introduction of exotic species include the increasing levels of international trade (increasing numbers of introductions), increased speed of transport of imported goods (enhanced survival of adventive organisms), and the difficulties of adequately inspecting containerized cargoes. The movement of people and goods has increased the rate of introductions by orders of magnitude, resulting in a trend towards the globalization of adventive species.

Table 3. Non-indigenous introductions on Garry oak in British Columbia

Species	Common Name
Coleoptera	
Otioryhnchus ovatus (L.)	Strawberry root weevil
Otioryhnchus singularis (L.)	Clay-colored root weevil
Otioryhnchus sulcatus (Fab.)	Black vine weevil
Phyllobius oblongus (L.)	A weevil
Strophosoma melanogrammum Foerster	A weevil
Lepidoptera	
Archips rosana (L.)	European leafroller
Ditula angustoriana Haworth	A leafroller
Choristoneura rosaceana (L.)	Obliquebanded leafroller
***Operophtera brumata* (L.)	Winter moth
†*Lymantria dispar* (L.)	Gypsy moth
Pandemis cerasana Hubner	A leafroller
Spilonota ocellana (Denis & Schiff.)	Eyespotted budmoth
Homoptera	
Moritziella corticallis (Kaltenbach)	An oak bark phylloxeran
***Phylloxera glabra* (Heyden)	Oak leaf phylloxeran
Hymenoptera	
***Neuroterus saltatorius* Edwards	Jumping gall wasp

** Denotes species that has caused serious defoliation

† Species has been recovered from Garry oak but is not established in British Columbia

Most countries have plant quarantine agencies, part of whose job is to monitor the influx of non-indigenous organisms, identify the pathways by which they are entering, and attempt to prevent their entry. The interception records of such quarantine agencies serve as a source of information on what is entering a country; collections made by government agencies and academics provide an indication of what species have established (populations of a species that persist in a new habitat). Unfortunately, neither source provides a complete picture. Interception records only document the species recovered from those imports that are inspected, a small subsample of the total volume of imported goods. Inspection efforts are limited by finite resources. It is often not possible to identify to species due to lack of expertise, financial resources, or facilities to rear to adults. In some cases, stain fungi for example, we lack the diagnostic tools to make positive identifications.

Over the past 5 years our research has focused on non-indigenous species associated with solid wood packaging and dunnage. It has been focused in two areas: the detection of species at ports of entry prior to their establishment and surveys for the detection of non-indigenous introductions in natural forest ecosystems. The primary taxon of concern is the Coleoptera, especially those species living under the bark or in the wood, predominantly the bark and ambrosia beetles (Scolytidae), longhorned woodborers (Cerambycidae), and flat-headed woodborers (Buprestidae).

Interceptions

The recent discovery of established populations of the European pine shoot beetle (*Tomicus piniperda* (L.)) in eastern North America and the Asian longhorned beetle (*Anoplophora glabripennis* Mots.) in New York and Chicago has highlighted the risks of introduction posed by solid wood packaging associated with commodities which themselves cannot harbour exotic species. One aspect of our research has focused on determining the incidence and composition of the arthropod faunas associated with wood packaging. The arthropod fauna associated with containerized shipments of architectural stone from Norway and wire rope spools from Asia are documented below.

Dunnage Associated with Granite Blocks. In July 1998, live beetles were found associated with shipments of granite from Norway. The shipments had entered Canada at the port of Montreal and had been shipped by rail to Vancouver, where the containers were unpacked and the dunnage discarded. Green spruce bolts had been used to brace large granite blocks inside shipping containers. The intercepted dunnage was brought to the CFS quarantine facility in Victoria and held under containment for emergence of the arthropod fauna.

The results of these rearings were alarming. More than 2,500 adult insects representing more than 40 species of bark beetles, woodborers, and their associated parasitoids, predators, and scavengers were recovered from 29 log bolts (Fig. 1). At least three species of Scolytidae of quarantine significance (*Pityogenes chalcographus*, *Polygraphus poligraphus*, and *Ips typographus*) were recovered from these bolts (Table 4).

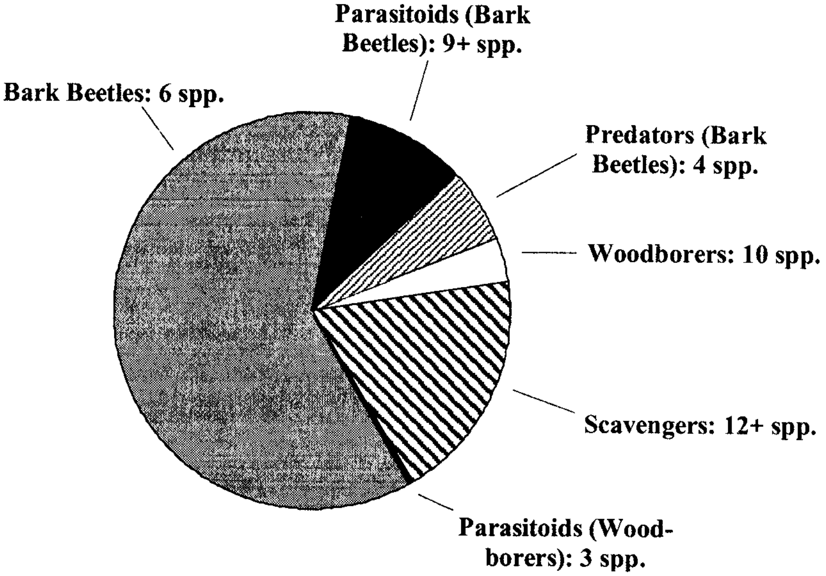

Figure 1. The composition of the arthropod community reared from spruce bolts used as dunnage in large granite block shipments from Norway.

Table 4. Bark and wood-boring beetles and wasps reared from intercepted spruce bolts from Norway used as dunnage; all rearings were done under containment in quarantine

Species and Family	No. of Individuals
Scolytidae	
Pityophthorus micrographus (Linnaeus)	942
Pityogenes chalcographus (Linnaeus)	284
Polygraphus poligraphus Linnaeus	207
Ips typographus (Linnaeus)	27
Crypturgus hispidulus Thoms.	16
Pityophthorus pityographus (Ratzeburg)	1
Cerambycidae	
Tetropium fuscum (Fabricius)	44
Callidium coriaceum Paykull	3
Molorchus minor (Linnaeus)	1
Pogonocherus fasciculatus (Degeer)	1
Semanotus undatus (Linnaeus)	1
Anobiidae	
Anobium sp.	10
Ernobius explanatus (Mannerheim)	4
Curculionidae	
Rhyncholus sp.	1
Melandryidae	
Serropalpus barbatus (Schall.)	7
Siricidae	
Sirex juvencus (Linnaeus)	21

Wire Rope Spools. Interceptions of *A. glabripennis* were first made in British Columbia in 1992. In that year, large numbers of newly emerged adults of *A. glabripennis* along with *A. nobilis* and *A. chinensis* were intercepted emerging from wood packaging in a container of pipe flanges. In 1995, adult *A. glabripennis* were again intercepted in a warehouse in greater Vancouver, this time apparently emerging from wood used to construct spools holding industrial wire rope. This led us to take a closer look at wire rope spools.

In 1997, 50 wire rope spools originating from China were obtained from various importers on Vancouver Island and the lower mainland of British Columbia. Many were empty discarded spools stored at import facilities and may have been in Canada for up to 2 years. The spools were disassembled and examined for evidence of woodborers. Twenty-four percent of the spools examined still contained live woodborers while a total of 31% of the spools had some evidence of past woodborer activity. Six species of longhorned woodborers (Cerambycidae), including *Monochamus alternatus*, *Hesperophanes* [=*Trichoferus*] *campestris*, *Ceresium flavipes*, *Psacothea hilaris*, *Megopis sinica*, and *Rhagium inquisitor*, and one species of Anobiidae, *Ptilineurus* sp., were obtained when wood from these spools was held in containment for adult emergence. The following year, an additional 16 newly arrived spools originating from China were sampled. Live insects were again recovered from 22% of the spools. There was often no visible external evidence of the presence of live woodborers in these spools: only 63% showed external signs of woodborer activity while all were found to have some evidence of past insect activity when disassembled.

Establishments

Our knowledge of non-indigenous species that have established is very limited; indeed, baseline data on the abundance and distribution of our native species is largely lacking. Our second area of research has focused on detecting non-indigenous species in forest ecosystems and determining their abundance relative to the abundance of our native taxa. These baseline surveys have been conducted using baited funnel traps and through rearing of naturally attacked host material.

Bousquet (1991) recently documented the composition of the Canadian beetle fauna. He recorded 7,436 species of beetles in the Canadian checklist, including 501 non-indigenous species (6% of the Canadian fauna). The highest proportions of non-indigenous species relative to the total provincial faunas occur in Newfoundland and the Maritime provinces (Fig. 2). Considerable variation is evident in the number of non-indigenous beetles found across Canada (Fig. 3), with the highest numbers occurring in Quebec, Ontario, and British Columbia.

When studies were initiated at forested locations around greater Vancouver in 1995, 135 species of Scolytidae and 145 species of Cerambycidae were known to occur in British Columbia (Bousquet 1991, Bright and Skidmore 1997). Five of the species included in the former family were previously established non-indigenous species, while no non-indigenous species of the latter family were known to occur in the province. To date, an additional five species of ambrosia beetles (Scolytidae) and one non-indigenous longhorned woodborer have been discovered in trap surveys. All of the newly discovered Scolytidae are confirmed as established, having been collected from multiple locations over at least three of the years in which sampling was conducted. The single cerambycid discovered was recovered from two locations in only one year.

These newly discovered introductions already comprise a significant proportion of the total bark and ambrosia beetle fauna trapped at some locations (Fig. 4). A similar pattern is evident when the scolytid fauna emerging from naturally attacked host material is examined (Fig. 5). Again, the majority of the emergent adults was non indigenous. Studies are currently being initiated to determine the biology and impacts of these recently introduced species.

The abundance of non-indigenous species relative to our native species of Scolytidae in both trap surveys and rearings from recently dead or dying native tree species indicate that these invasive taxa have successfully adapted to their new environments. At least two of the species of Scolytidae discovered to date are attacking hosts not utilized in their native ranges. One has established more than 1000 km north of its native range where representatives of our native genera do not occur, while the second breeds in conifers as well as species of deciduous trees in genera known as hosts in its native range.

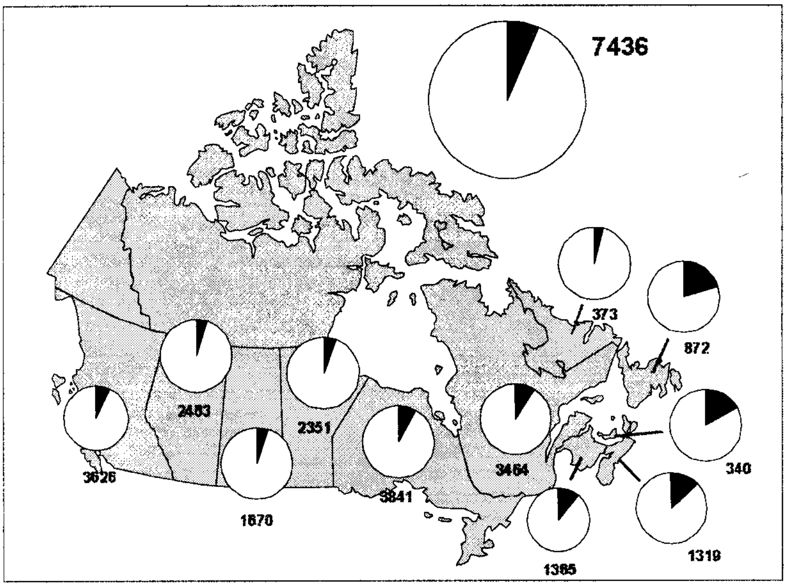

Figure 2. Total number of species of Coleoptera in Canada and the number of species present in each province (data from Bousquet 1991). The proportion of non-indigenous Coleoptera species present in each region is denoted in black. Numbers are the total number of Coleoptera species in each region.

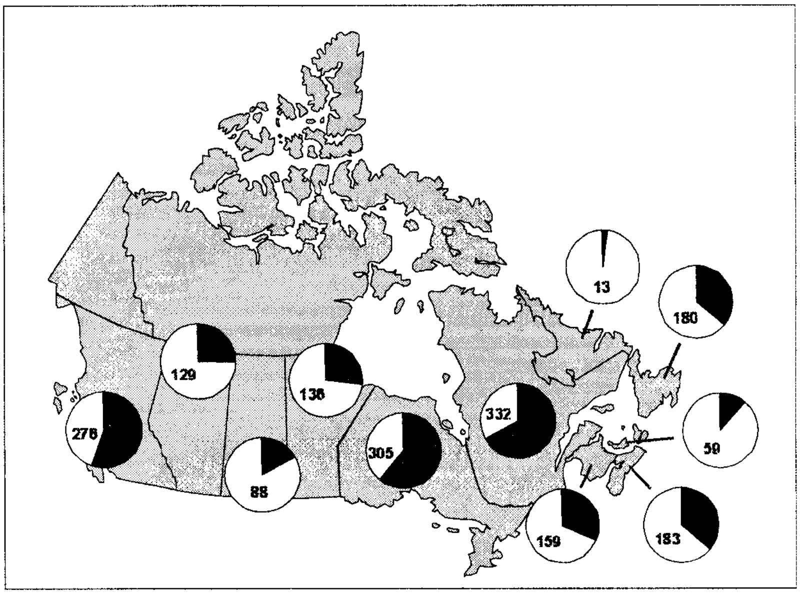

Figure 3. The proportion of the total number of non-indigenous Coleoptera present in Canada (n=501) found in each region of the country is denoted in black (data from Bousquet 1991). Numbers are the total number of non-indigenous Coleoptera species in each region.

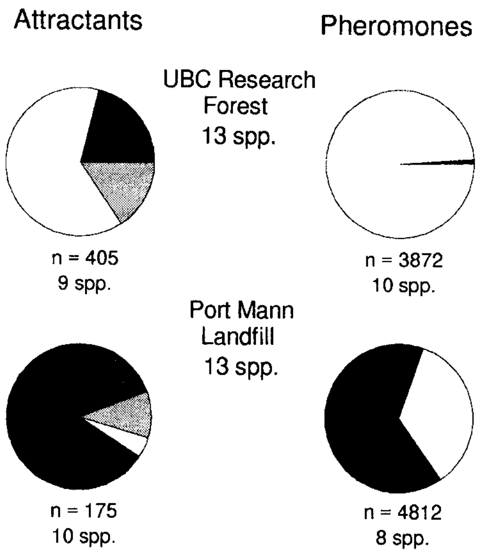

Figure 4. Relative abundance of native species (white), non-indigenous species recorded by Bousquet (1991) and Wood and Bright (1992) (grey), and non-indigenous species discovered in these studies (black) responding to traps baited with attractants (ethanol, α-pinene, dipentene, methyl salycilate) or pheromones (lineatin, sulcatol, chalcogran, ipsdienol) at two locations in the Fraser Valley, near Vancouver British Columbia between 16 March and 16 July 1999.

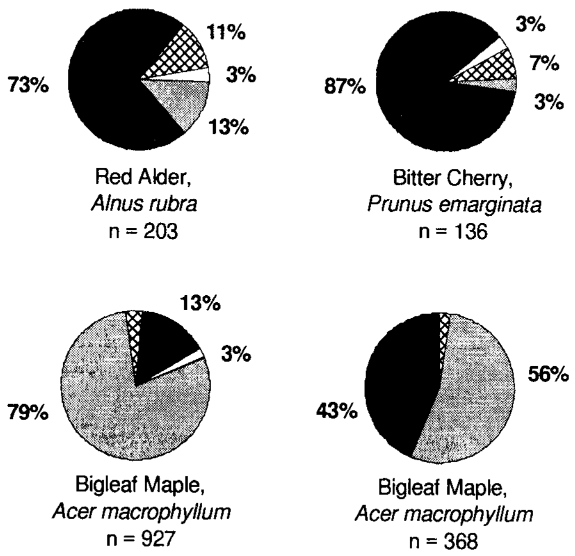

Figure 5. Relative abundance of native species (white), non-indigenous species recorded by Bousquet (1991) and Wood and Bright (1992) (grey), and non-indigenous species discovered in these studies (black) reared from the noted field attacked tree species collected at the Port Mann Landfill site, Surrey, B.C. in 1997. Cross hatching indicates the presence of males of two non-indigenous xyleborine ambrosia beetles that could not be identified accurately and thus could not be accurately placed in any category.

Summary

We have provided a brief review of the status of non-indigenous species as pests in the forests of Canada. Recent research findings related to the introduction and establishment of invasive bark and woodboring insects associated with solid wood packaging that demonstrate that non-indigenous species continue to arrive on our shores and establish in our forests as a consequence of international trade are presented. The recent introductions discovered during these studies in a limited area of southwestern British Columbia have doubled the number of non-indigenous Scolytidae known to be established in the province. Interceptions from solid wood packaging indicate that species will likely continue to accumulate in the near future. Because we lack an *a priori* knowledge of the biology and ecology of these invasive species in their new environments, we cannot predict the potential impacts they may cause, either individually or cumulatively, on our forest ecosystems.

To address the issue of non-indigenous introductions and their impacts we need to:

(1) increase our capability to quantify (more than monitor) the flow of insect species (and other organisms) in and out of the country and within national boundaries (e.g., across ecosystem boundaries);
(2) improve our capacity to identify species;
(3) study the establishment and distribution of non-indigenous species in native ecosystems to better understand their effects on ecosystem function;
(4) provide advice to government agencies responsible for plant quarantine issues; and
(5) foster international cooperation to share information on species of concern (specimen exchange, cooperative research, shared databases).

It is our hope that such efforts coupled with the adoption and enforcement of new regulations on solid wood packaging will stem the flow of invasive bark and woodborers into North American forests.

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