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Monitoring and control of *Ips sexdentatus* Boerner using synthetic pheromones (*)

Abstract - This study examines the possible methods for *Ips sexdentatus* Boerner control in the more general context of integrated protection of woodland areas. In consequence of the fire this bark beetle showed important gradations in the Natural Reserve of Monte Alpe (Lombardy, Northern Italy). According to biological observations, carried out in 1991-92 at different altitudes (800-1200 m), the species accomplished 3 flights; the first during May-June, the second in August, and the third in October. The research focussed on the identification of some components of the pheromonic mixture, and on the possibility of using attraction substances to stimulate the aggregation effect. The «radiator» traps demonstrated a greater effect than the «tube» traps in attracting the Scolitidae. Pheromone baited tree-trunks showed the same level of efficacy as the «radiator» traps, and a much higher efficacy to that of tree-trunk traps without pheromone. The (+) α-phellandren-8-ol and (–) α-phellandren-8-ol were tested to evaluate the attraction of *I. sexdentatus*. The new molecules when used alone do not have an attractive effect, but when used in combination with racemic ipsdienols have a synergic effect.

Riassunto - *Monitoraggio e controllo di* Ips sexdentatus *Boerner con l'uso di feromoni sintetici.*

Il lavoro prende in esame le possibilità di controllo di *Ips sexdentatus* Boerner (Coleoptera: Scolytidae) nel più generale contesto della protezione integrata del bosco. Lo scolitide, poco noto in Italia, convive normalmente con i suoi ospiti senza causare danni; tuttavia, in annate favorevoli, od in presenza di fattori scatenanti come l'incendio sviluppatosi nella riserva naturale del Monte Alpe (Lombardia, Italia settentrionale) nell'inverno 1989-90, possono verificarsi importanti gradazioni che interessano anche piante in buone condizioni vegetative. Le osservazioni biologiche, condotte negli anni 1991-92 a diverse fasce altimetriche (800-1200 m), hanno evidenziato come la specie compia tre voli, il primo a maggio-giugno (individui svernanti), il secondo in agosto

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(prima generazione dell'anno), il terzo ad ottobre (seconda generazione dell'anno). La ricerca si è concentrata sull'identificazione di alcuni componenti la miscela feromonica della specie e sulle possibilità d'uso di sostanze attrattive stimolanti l'effetto di aggregazione per la lotta contro questo insetto. Le trappole «a radiatore» hanno dimostrato maggior attività di quelle a «tubo» nel catturare lo scolitide. A loro volta tronchi esca innescati con feromone hanno manifestato la medesima attrattività delle trappole a «radiatore» ed un'efficacia molto superiore rispetto a quelli senza feromone. Sono stati testati, per valutarne l'attività nei confronti di *I. sexdentatus* (+) α-fellandren-8-olo x (–) α-fellandren-8-olo. Si è evidenziato come le nuove molecole, di per sé poco attrattive, possiedano un effetto sinergico, quando usate in combinazione con ipsdienolo racemo e quindi entrino con tutta probabilità a far parte del bouquet feromonico della specie.

Key words: *Ips sexdentatus*, synthetic pheromones, monitoring and control.

INTRODUCTION

The bark beetle *Ips sexdentatus* Boerner is distributed all over Europe; in the East it follows the *Picea orientalis* L. in the regions were it is found, in the West it mainly attacks the species of the genus *Pinus* and in particular *Pinus nigra* Arn., *P. sylvestris* L., *P. leucodermis* Antoine, *P. pinaster* Aiton. Its presence has sometimes been noted on larch trees (*Larix decidua* Miller).

The insect prefers warm climates, where it is considered to be a serious pest, mainly due to the capability of completing up to 5 generations a year. In Europe, it is frequent above all in artificial plantations of *Pinus*, where it normally occurs without reaching serious levels of damage, but in favourable years or in the presence of inciting factors, large increases in the population numbers can take place leading to devasting gradations due to the possible attacks on seemingly healthy plants. In particular, recently, this event took place with worrying proportions in nearby France favoured by the warm, dry climate of 1989-90, to the extent that the French Forestry Service decreed a 1st grade of risk for this insect all over the country. In the Mediterranean area it is the 2nd most important pine pest, surpassed if only by a small degree, by processionary caterpillar of the pine *Thaumetopoea pityocampa* Den. & Schiff.. Similar situations took place in Portugal (Paiva et al., 1988) and in Greece (Chararas, 1962). As far as Italy concerned, *I. sexdentatus* is present all over the Appenine and Mediterranean areas, with sightings also in Sardinia, without ever having caused big problems.

The study area, in the Natural Reserve of Monte Alpe, in the province of Pavia, is a very different case, where, after a fire, a high number of deteriorat-

ing hosts were available for reproduction. The presence of *T. pityocampa* and Scolitidae of the genus *Tomicus* Latreille (= *Blastophagus* Einchhoff) must also be included in the context, since they contribute to the state of stress, favouring the attack of *I. sexdentatus*. In this way, the population was able to reach such large numbers in a short period of time to allow even major attack on neighbouring areas, occupied by apparently healthy trees. All this has caused a dangerous gradation which may lead to the total destruction of the pines of the Reserve.

The case of Monte Alpe is unique in the outline of the Natural Reserves of Lombardy. It is, in fact, an almost completely artificial environment; mostly made up of black pine woods (*Pinus nigra*), planted starting from 1936, the scientific interest of which is connected essentially to the presence of populations of *Formica lugubris* Zett. originating from the Alps and transplanted in locus to control the *Thaumetopoea pityocampa* Den. & Schiff. (Pavan, 1960).

The reserve covers an area of 327.56 hectares, at an altitude between 850 and 1259 meters, and also includes some old coppices, in part mountain and sub-mediterranean oak-woods, and in part more recently re-planted woodland areas, with various species of conifers, and 28 hectares of meadows, now abandoned.

The predominating lithological substratum is made up of regular alternations of limestone and marble, with local clayey layerings. To these components we must also add the superficial deposits, which include the usual coulters of moderate thickness, and the accumulations of debris and landslides.

The research was aimed at the bases of the insect ecology in forestry environments in Lombardy, for the design of a comprehensive pest management program. Emphasis was given to the use of attractive substances, stimulating the aggregation effect which at present is the most realistic strategy for Scolitidae control in a forestry environment.

Little is known about aggregation signal of *Ips sexdentatus*; in 1983 Franke and Vité highlighted the evidence that ipsdienol is involved in the aggregation signal and that the male sends it out in a cluster form. On the other hand, Klimetzek and Vité (1986) proved that combination of 2-methyl-3-buten-2-ol and ipsdienol is considered to be connected to the long distance signal, while the chemical mediator of the short distance signal still has to be identified. The presence of terpen alcools has been discovered among the molecules produced by the species, which are considered to be involved in the aggregation signal. Among these, the α-phellandren-8-ol (p-mentha-2,5-dien-8-ol) (Franke et al., 1986) is particularly interesting. There are several problems regarding the production of this substance, as suggested by Mori & Igarashi (1988). A variant of the synthesis method has been developed for research purposes, making available the two (+) α-phellandren-8-ol and (–) α-phellandren-8-ol.

MATERIALS AND METHODS

Biological cycle in the experimental environment

Weekly controls were carried out from April to October checking the attacked plants and controlling the pheromone and the tree-trunk traps. Investigations were carried out in late Autumn and at the end of the Winter to be able to identify the overwintering life stages. The flight curves were reconstructed using the data of six traps baited with Pheroprax®([1]).

The observations regarding the course of the cycle were carried out by means of a gradual peeling of the trees at ground level attacked by this bark beetle. During 1992 a Summer generation of *I. sexdentatus* was bred in climatic cells, at a constant temperature of 24 °C on pieces of black pine wood collected when already infested by eggs and larvae.

Ecological and ethological aspects

The study was carried out in 1991-92, and the following three tests relative to monitoring and control were conducted.

1) Evaluation of the efficacy for *I. sexdentatus* of the pheromone traps set up for *I. typographus*. The traps, three of which were the «tube» kind and another three were «radiator» traps([2]), were positioned on wooden supports, with a 15 meter space between each, and with copies at a distance of 400 meters. This set-up corresponds to the one usually indicated in literature.

2) Verification of the attraction combination of pheromone and tree-trunk traps and their efficacy compared to the pheromone traps. In this test three experiments were compared, repeated three times, composed of:

— a tree-trunk trap treated with deltamethrin (Decis®([3])) at a concentration of 1,25 ml/l, being careful to distribute 0.2 l of preparation per linear meter of the tree-trunk trap;

— a tree-trunk trap prepared as the previous, belonging to the same tree, baited with pheromone;

— a Rochling® trap supplied with pheromone.

In the tests 1) and 2), carried out in 1991, the aggregation pheromone Pheroprax® was used being synthetic attraction mixture for *I. typographus* made up of ipsdienol + 2-methyl-3-buten-2-ol + (S)-cis-verbenol. This is the only pro-

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([1]) Pheroprax® trade mark by Celamerk Germany.

([2]) The «tube» trap trade mark by Booregard Norwey; the «radiator» traps trade mark by Rochling Germany (tests 1 and 2) or by Theysohn Germany (test 3).

([3]) Decis® trade mark by Roussel-Hoechst.

duct on sale at the moment which shows any activity, though limited, towards *I. sexdentatus*.

3) Identification test of the aggregation signal of *I. sexdentatus*. At the moment there is no pheromone on sale for *I. sexdentatus*, however, research on the aggregation signal of this insect has been going on for several years.

The test, carried out in 1992, repeated four times, used 20 «radiator» traps by Theysohn®, baited with the following compounds:

A) (–) α-phellandren-8-ol (1 ml of solution in ethanol containing 25 mg of a. i.).

B) (+) α-phellandren-8-ol (1 ml of solution in ethanol containing 25 mg of a. i.).

C) 15 mg racemic ipsdienol + 1200 mg 2-methyl-3-buten-2-ol.

D) (+) α-phellandren-8-ol + 15 mg racemic ipsdienol + 1200 mg 2-methyl-3-buten-2-ol.

E) (–) α-phellandren-8-ol + 15 mg racemic ipsdienol + 1200 mg 2-methyl-3-buten-2-ol.

Ipsdienol and 2-methyl-3-buten-2-ol were evaporated separately from the glass capillary tubes (internal diameter of 0.9 mm) placed in test-tubes of 100 mm, closed with caps with four holes of 1 mm. With this preparation the release of ipsdienol is of 30 μg/h, that of 2-methyl-3-buten-2-ol of 400 μg/h (Bakke, 1978).

The dispenser of the α-phellandren-8-ol is made up of a plastic container hermetically connected to a Millipore® filter maintained wet by means of dropping, through which the evaporation of the solution is carried out. This enables a release of 35 mg of solution / day at a temperature of 42 °C, established by the difference in weight.

RESULTS AND DISCUSSION

Biological cycle

The growth cycle of *I. sexdentatus* is schematized in fig. 1. The overwintering took place (regardless of the birth places) on old trunks and on trees suitable for reproduction. The insect was found in fact both as an adult and in pre-imaginal stage in the Winter of 1992-93. This is in contrast to the previous Winter, when only young, immature adults were found, characterized by the pale color of the cuticle.

I. sexdentatus adults are more sensitive to the cold than pre-imaginal life stages. The adult activity is considerably reduced when the temperature drops below 7 °C, while at 4 °C the activity stops completely, and the adults enter the overwintering phase. Big differences in temperature (from +18 °C to –10 °C) can cause the death of more than 50% of the population.

Hibernating adults gradually leave the shelter in Spring, when the tempera-

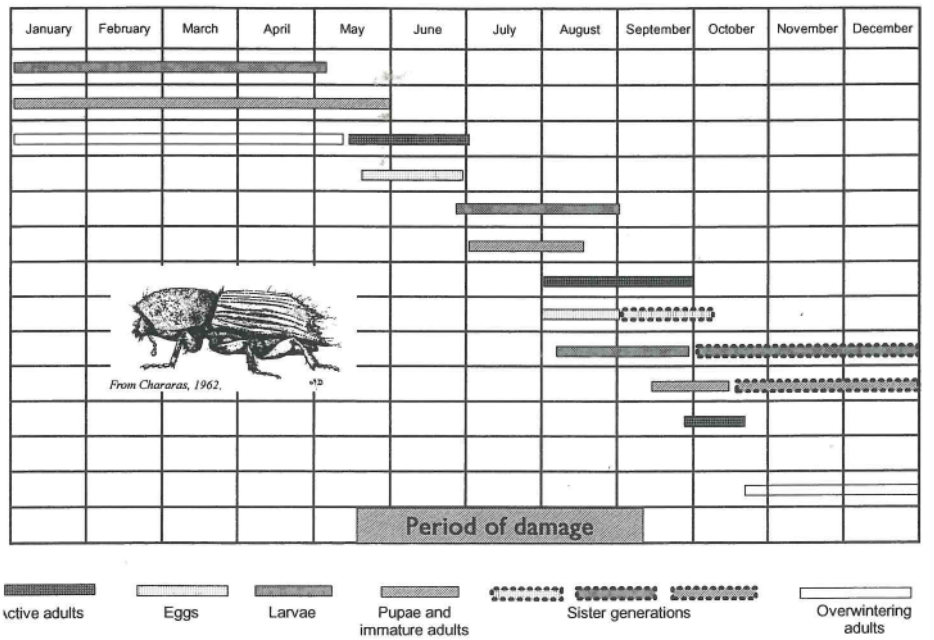

Fig. 1 - Annual cycle of *Ips sexdentatus* in the natural reserve of Monte Alpe (Pavia).

ture is at least 18 °C. The effect of the temperature and the wind has been ascertained by a simple observation of the traps on days which are ideal for flight and on days which do not permit the flight. Similarly, the rain causes immediate standstill of flight. The weather pattern has a graduating effect on the flight. The date of the emergence from the shelter can reasonably be matched with that of the beginning of the captures in the traps, which took place on the 27th of June 1991 (fig. 2), and on the 20th of May the following year (fig. 3). The considerable difference can be attributed to the unfavourable weather patterns of 1991. In fact on the 18th of April there was a late snow fall, and the unfavourable weather continued until April the 29th, heavily conditioning the insect.

In a period of 1-5 days the male of *I. sexdentatus* chooses the tree on which to settle and starts to dig a nuptial chamber. Once this operation is finished, the arrival of the females can be observed. According to Chararas (1962), these are fertilized before the depositing tunnel is begun. In the initial phase of the diggings (about 5 cm) the presence of the males moving backwards along the tunnels was observed. It is known that the females are fertilized several times by different males; which enter through the ventilation holes of the mother tunnels, but this happens only at a later stage. In the initial stage the males participate by transporting and removing the wood dust linely clowed in the formation of the mother tun-

nels. From the data on the composition of the families at the beginning of the attack, we can see how the sex-ratio is of 2 to 3 females per male (in the following attacks it is often impossible to establish the proprietor of the tunnels because of their density), even if there can be samples with a larger number of females present.

It was also observed that the form of the mother tunnels is characteristic, and looks like a candelabrum. On either side of these, the female lays 19 to 20 eggs starting at a distance of 1 cm from the nuptial chamber, in special cells, built using their jaws, and closed with wood dust linely clowed and droppings. The length of the mother tunnels varies in proportion to the density of the families on the host. A minimum length of 10 cm and maximum length, on rare occasions, of 64 cm were observed. A characteristic is the previously mentioned ventilation holes.

Once the eggs have hatched, the larvae start to dig tunnels perpendicular to the mother tunnels, at first completely separate one from another, then in close contact because of the complete destruction of the host-tissue. Pupation-takes place in the same environment, and immature adults emerge after about ten days. These complete their development on the original host in about 15 days.

The duration of a generation varies above all, due to the temperature considering the «thermal buffering» effect of the tree.

The observation of the ratio of males to females captured in various phases of flight is interesting. Males and females were separated by means of dissection (100 insects were taken at random when samples were numerous). The ratio between the capture of males and females obtained in this way was 1 to 1.5, confirming the

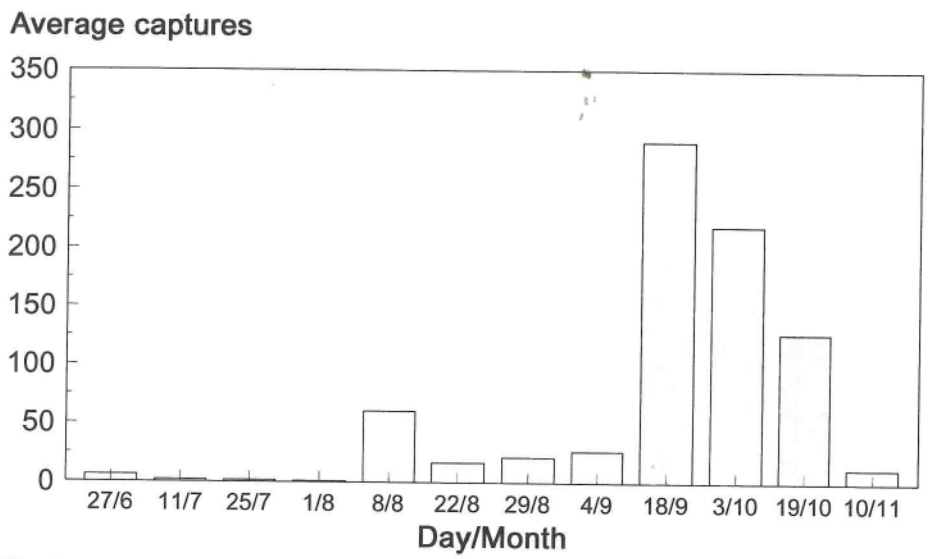

Fig. 2 - Flight curves in 1991 (pheromone used: Pheroprax®): average capture rate.

diverse attractivity of the pheromone on the two sexes (Serez & Schönherr, 1985). The expected sex-ratio, however, is 1 to 1 (Chararas, 1962).

As seen in the graph of fig. 4, the capture of males has two peaks; the first at the beginning of flight and the second a month later, and points out a certain level of protandry of the species.

The new adults give way to a second generation in the Monte Alpe region, which begins in August and ends in September-October with the flight of the adults towards the overwintering places. This event involves only a part of the individuals since a great number of these overwinter on the host on which they have developed. A third generation can be studied if conditions are favourable (Chararas, 1962), but this was not noted in this period in the observed environment.

The cycle is complicated by the presence of sister generation. In fact, the females which have already layed eggs, can lay a second or even third time on another host plant of the same species (Chararas, 1962). This characteristic, which is presented by Jactel & Lieutier (1987) as an answer to the density of attacks on a single host, leads to an overlapping of flights. The sister generations are particularly evident in the second flight, which is numerically more consistent. The larvae found at Winter's end are probably due to sister generations of the August flight.

In the pine woods of Monte Alpe, typical star-shaped galleries (formed by sister generations), short and with few eggs, were rarely found. Considering the mass of potential hosts, this seems to confirm the results of the two above named researchers indirectly.

Ecological and ethological aspects

The presence of the insect was noted only on black and silver pine, while the larch was never attacked.

As far as the resistance of the host is concerned, the author frequently observed trees that had been attacked but not colonized by Scolitidae. These trees were characterized by garish, resinous pads in the areas of attempted entry and by the presence of very few dead insects enclosed in the resin, sign that *I. sexdentatus* abandons the host plant once it realizes that it cannot be attacked. The considerable ability to disperse permits the insect to identify damaged woodland areas with ease, and has a great importance in the dynamics of the population. Once *I. sexdentatus* has started a gradation, thanks to its characteristics, it can originate new infestations in an area of several square kilometers. This is the reason why *I. sexdentatus,* which had a focus point at a distance of 2 km, colonized, in July 1992, several cut trunks which had not been quickly cleared.

The number of susceptible hosts is strongly connected to a series of factors, in particular:

— the inadequacy of the chosen tree species for the environment, revealed in the phase of maturity of the woods by means of a rapid decline, greatly favouring the insect;

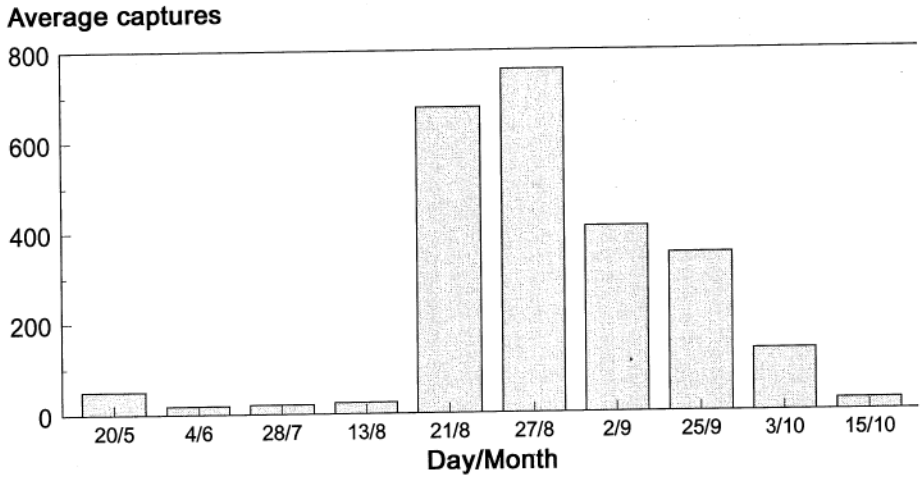

Fig. 3 - Flight curves in 1992 (pheromone used: Pheroprax®): average capture rate.

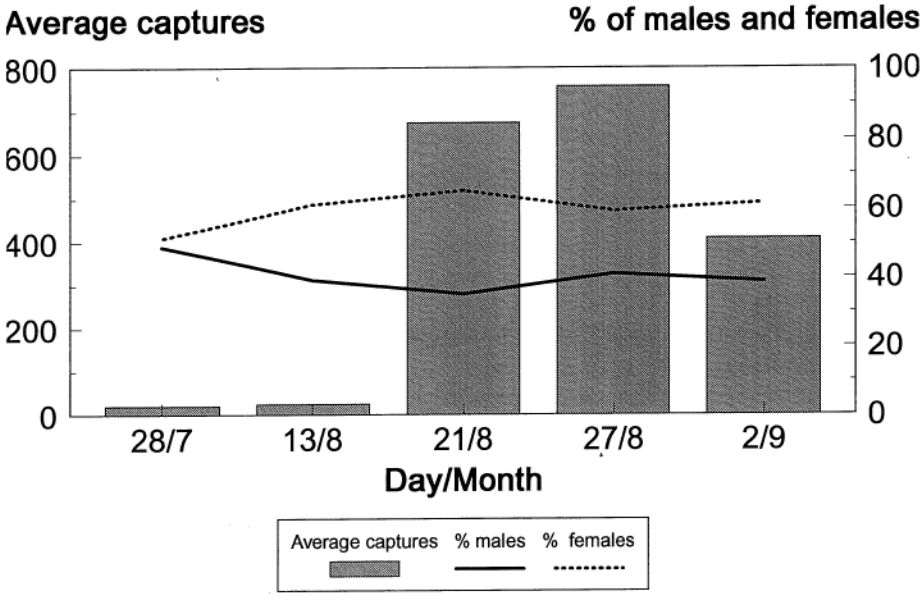

Fig. 4 - Average captures and percentage of males and females during flight.

— the unfavourable climatic pattern of the past years, characterized by warm Winters, late snow-falls and dry Summers, which heavily conditioned the hosts, lessening their resistance.

— particular events, such as a fire, which had a great importance for the gradation in the pine-woods in the Natural Reserve of Monte Alpe. After the fire in the Winter of 1989-90, in fact, there were a great number of dead trees with characteristics which allowed the development of the insect, and also a number of weakened trees three times larger. The delay in the clearing of this material gave way to an uncontrollable growth in numbers of Scolitidae. The subsequent complete cutting of the dead plants then opened clearings permitting the immediate colonization of the plants on the borders of these clearings. These plants were the most exposed to the actions of the wind and the weight of the snow; causing the breakage of branches and roots, as well as burning caused by the sun and the dryness. It is important to underline that the microclimate of the periferic areas is less humid than that in which the plants had been previously. Their foliage is also limited to the highest part of the trunk; the lower branches dying as they grow because of the shading due to the overcrowding of the installation.

Defoliators and phloem-borers insects were also very important in this study, above all *Thaumetopoea pityocampa* and *Tomicus piniperda* (L.).

According to the level of damage caused by the fire the trees were divided into:

— apparently healthy trees, perfectly green;
— trees suffering from evident physiological problems.

The level of resistance of the two categories was very different, the second being easily attacked and colonized by *I. sexdentatus*. The plants of the second category did not reveal any resistance. The plants of the first category were attacked during the gradation, but the attack was not always successful. When infested the pines of the first category are only attacked by the Summer generation, which is initially confined to the highest parts of the tree and only later covering the whole plant.

The success of the colonization is indicated by the presence of abundant humid wood dust linely clowed which in any way cannot be immediately identified because the laying of the eggs, especially during the Spring attack, and also in the Summer attack of healthy plants, takes place in the higher parts of the host.

Evaluation of new synthetic attractive mixtures

In the Summer of 1992 we used traps baited with substances thought to be involved in the transmission of the aggregation signal of *I. sexdentatus*. Table 1 shows that, if evaluated singularly, the new molecules tested have almost no activity. The result of the combination with ipsdienol is very different. In this case

Table 1 - Average values of captures (± S.E.) of I. sexdentatus *in the four traps baited each with one of the five different test substances and significant differences in the average captures (Duncan's multiple range test p < 0.05).*

Test	13/VIII captures ± S.E.	21/VIII captures ± S.E.	27/VIII captures ± S.E.	2/IX captures ± S.E.
A	0.75 ± 0.48 a	1.25 ± 0.75 a	3.25 ± 1.97 a	0.75 ± 0.47 a
B	0.25 ± 0.25 a	0.75 ± 0.48 a	1.00 ± 0.70 a	1.75 ± 1.03 a
C	51.00 ± 25.67 a	172.75 ± 41.94 b	95.25 ± 26.67 a	8.25 ± 3.30 a
D	0.25 ± 0.25 a	93.75 ± 37.97 ab	318.25 ± 93.55 b	183.00 ± 68.21 b
E	382.25 ± 129.70 b	368.75 ± 68.46 c	172.00 ± 69.33 ab	16.50 ± 8.39 a

they seem to show a synergic effect similar to that noted for *I. typographus*, which needs (S)-cis-verbenol + ipsdienol in the aggregation pheromone (Bakke et al., 1977).

On the other hand, there are still doubts on the exact optical configuration of the new molecules used even if (–) α-phellandren-8-ol seems to be the most active configuration. The evident differences between the captures are probably due to the characteristics of the dispenser used.

Table 2 underlines how (–) α-phellandren-8-ol tends to increase the captures of females compared to ipsdienol. The relationship M/F of 1 to 2.1 shown by the captures using ipsdienol is already high, even in comparison with the ratio of 1 to 2.3 observed by Klimetzek & Vité (1986).

Table 2 - Sex ratio of I. sexdentatus *with pheromones tested. (Test A and B collected too few examples to give reliable data).*

Test	C	D	E
males/females	1/2,1	1/1,9	1/2,5

Comparison between traps

Attention was given to «radiator» and «tube» traps. The first trap simulates the shape of a colonized trunk, the second is simply based on practical standards. In both cases the colour used was black, and in both cases the same pheromone (Pheroprax®) was used. Table 3 shows the results of the experiment carried out in 1991.

Table 3 shows significant differences between the two kind of traps. The «ra-

Table 3 - Average values of capture (± S.E.) with the two different kinds of traps.

Date	«Tube» trap captures ± S.E.	«Radiator» trap captures ± S.E.	Statistical significance
8 VIII	27.70 ± 2.33	58.00 ± 4.36	$p < 0.01$
22 VIII	6.66 ± 0.88	20.66 ± 5.66	N.S.
4 IX	0.00	28.33 ± 8.57	$p < 0.05$
18 IX	0.00	52.00 ± 17.60	$p < 0.05$
3 X	0.33 ± 0.33	385.33 ± 120.83	$p < 0.05$
11 X	0.00	289.00 ± 82.65	$p < 0.05$

diator» traps work much better than the «tube» ones, even if it is not clear why. This may be due to the circulation of air inside this model, which facilitates the dispersion of the pheromone. On the other hand it could be a visual stimulus, because the diameter of the pipe is less than 20 cm, while *I. sexdentatus* prefers large trunks with thick bark.

Table 4 - Average values of capture (± S.E.) carried out using the three methods discussed in the text. Different letters indicate significant differences for $p < 0.05$ (Duncan's multiple range test).

Date	Tree-trunk trap captures ± S.E.		Pheromone baited tree-trunk trap captures ± S.E.		«Radiator» trap captures ± S.E.	
3 X	34.66 ± 18.46	a	481.70 ± 18.44	b	499.30 ± 65.82	b
11 X	19.00 ± 9.85	a	134.00 ± 24.25	b	173.00 ± 44.21	b

Trunk traps and their attractivity compared to that of artificial traps

A comparison was made in 1991 to evaluate the activity of trunk traps, trunk-traps baited with pheromone and «radiator» traps (table 4), three replicates per trap.

Table 4 shows that the number of the bark beetle captured in the tree-trunk trap is smaller and significantly different from the number of adults caught in pheromone baited tree-trunk trap and in «radiator» trap. However, there was no significant difference between the latter traps. This result has a great importance if we consider the possible cost of the traps, used for *I. sexdentatus* control in large areas.

CONCLUSIONS

The observations made clear that *I. sexdentatus* accomplishes three flights: the first in May-June (overwintering individuals), the second in August (first generation of the year), and the third in October (second generation of the year). Sister generations were also observed during the August flights, even if rarely. All this leads to a considerable aggressiveness of the species all year.

The tests carried out to identify adequate control methods regarding the available traps use of traditional trunk-traps or in combination with the pheromone of aggregation, have indicated that these methods can not be proposed for mass-captures purposes. If we consider the traps, the «radiator» kind showed a greater efficacy compared to the «tube» kind. This is probably due to the better internal air-circulation, which enables the dispersion of the pheromone over greater distances.

The test with trunk traps have underlined how these, when synergized with pheromone, capture as many insects as the normal traps. Moreover, these traps are cheaper than the «radiator» and «tube» traps and can be recommended for pest management purposes.

The main problem regards the activity of the pheromone used, in particular those of *I. typographus*, which is therefore not specific for *I. sexdentatus*.

Both (+) α-phellandren-8-ol and (–) α-phellandren-8-ol increase considerably the attractiveness of the single ipsdienol when used combined with the latter compound. The observations carried out regarding the optical configuration a-phellandren-8-ol do not permit the identification of the most efficacious one.

Because the identification of the aggregation pheromone of the specie is not yet complete, the proposed methods can, at the moment, be evaluated only for monitoring.

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