UNIVERSITA' DEGLI STUDI DI PADOVA

SCUOLA DI DOTTORATO DI RICERCA IN
SCIENZE DELLE PRODUZIONI VEGETALI
INDIRIZZO PROTEZIONE DELLE COLTURE - CICLO XXIII
Dipartimento di Agronomia Ambientale e di Produzioni Vegetali

The engraver beetle *Ips acuminatus* in the south-eastern Alps
Life traits and population dynamics

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DATA CONSEGNA TESI
31 gennaio 2011

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To Andrea O. and Luigi C.

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Riassunto e obiettivi del lavoro di ricerca

Il Coleottero Scolitide *Ips acuminatus* (Gyllenhal) (Coleoptera: Curculionidae, Scolytinae) ha recentemente causato estese e gravi infestazioni in diverse zone distribuite sull'arco alpino e, tra queste, la valle del Boite (Dolomiti).

Lo scolitide, per lungo tempo considerato di scarsa rilevanza economica, è stato recentemente incluso tra le dieci specie di insetti xilofagi più dannose in Europa in quanto sempre più frequentemente associato a fenomeni di elevata mortalità del pino silvestre.

È noto che fattori abiotici quali elevata temperatura e stress idrico possono scatenare gravi infestazioni di *I. acuminatus*: per questo motivo è probabile che i cambiamenti climatici in atto aumentino i rischi cui possono essere soggette le pinete di pino silvestre, per l'attività sia dell'insetto da solo sia in associazione con parassiti opportunisti (secondari). Le gravi infestazioni segnalate negli ultimi anni sia sulle Alpi occidentali che su quelle orientali sembrano confermare tale ipotesi.

I. acuminatus è specie ad areale euroasiatico ed è uno dei più comuni organismi xilofagi legati al pino silvestre (*Pinus sylvestris*). L'insetto è poligamo e in un sistema di gallerie si possono trovare fino a 12 femmine associate a un singolo maschio. Le gallerie materne si irradiano da un vestibolo centrale scavato dal maschio, determinando un caratteristico 'sistema a stella'. Le uova sono deposte lungo entrambi i lati della galleria materna e le larve si sviluppano in brevi gallerie scavate nel floema, essendo la specie fleomicetofaga e comunemente associata a specie fungine funghi quali *Ophiostoma brunneo-ciliatum* e *Ambrosiella macrospora*. Prima di emergere dal materiale infestato, i giovani adulti necessitano di un periodo di maturazione che svolgono nutrendosi del floema situato nelle immediate vicinanze delle gallerie larvali e delle celle pupali in cui si sono sviluppati. A termine del periodo di maturazione, gli adulti sfarfallano e colonizzano i rami e la parte superiore dei fusti del pino silvestre, in quanto prediligono le parti con corteccia di spessore non superiore a 4-5 mm.

Fonti bibliografiche riportano la specie come monovoltina in Scandinavia e bivoltina in Europa meridionale. *I. acuminatus* sverna allo stadio adulto principalmente nel materiale infestato in cui si è sviluppato; per le popolazioni nordiche la lettiera sembra costituire un ulteriore sito di svernamento. I primi voli primaverili hanno luogo quando la temperatura dell'aria raggiunge i 14 -16°C, ma il picco dei voli si verifica quando questa supera i 18°C. Come molti altri scolitidi che adottano la strategia dell'attacco massale al fine di vincere le difese dell'ospite, le infestazioni di *I. acuminatus* si manifestano con attacchi diffusi e concentrati su un numero variabile di piante vicine tra loro, dando luogo a tipiche infestazioni definite a 'macchia di leopardo'.

Durante il lavoro di ricerca, condotto in concomitanza al verificarsi di una grave pullulazione dello scolitide nelle Alpi sud-orientali, si è cercato di chiarire diversi aspetti poco noti della biologia dell'insetto, dedicando particolare attenzione a:

- voltinismo, fenologia e performance riproduttiva considerate in relazione a temperatura e densità di colonizzazione, allo scopo di determinare come questi fattori possano influire sulla performance e sulla dinamica di popolazione dell'insetto nel quadro del cambiamento climatico;
- dinamiche spazio-temporali dei nuclei di infestazione di *I. acuminatus* durante le diverse fasi della pullulazione, al fine di prevedere il grado di attività e il possibile comportamento futuro dell'insetto;
- impatto di tagli fitosanitari sulle dinamiche di popolazione ed effetti nel limitare i danni arrecati alle pinete, allo scopo di stabilire criteri di valutazione utili nella gestione dei boschi infestati;
- complesso di nemici naturali associati a *I. acuminatus*, al fine di determinare quale ruolo e importanza possano avere parassitoidi e predatori sulla dinamica delle infestazioni.

I risultati relativi a ognuno degli aspetti sopra elencati sono in diverse fasi di pubblicazione in quattro articoli, corrispondenti a capitoli che compongono la presente tesi (dal II al V). Il primo capitolo (Introduzione), contenente una sintesi del lavoro svolto e parte dei risultati preliminari, è costituito da un articolo recentemente pubblicato (Forest@ 2010, vol. 7: 259-267).

Summary and objectives of the research work

In the last years extensive outbreaks of the pine engraver beetle *Ips acuminatus* (Gyllenhal) (Coleoptera: Curculionidae, Scolytinae) have been reported in many Scots pine (*Pinus sylvestris*) forests in the Alps including the valley of Boite (Dolomites).

The beetle has been considered for a long time of minor economic importance, but recently it has been included among the ten most damaging wood boring insect in Europe as a result of the increasing tree mortality caused by this species. Extrinsic factors such as high temperature and drought are known to trigger outbreaks of *I. acuminatus* and it is likely that climate change may aggravate the threat to pine forests, either with the beetle alone or in combination with secondary pathogens and pests, as the recent infestations seem to confirm.

I. acuminatus is one of the most common bark beetles infesting Scots pine (*Pinus sylvestris*) in Eurasia. It is a polygamous species with up to 6-12 females joining each male within the gallery systems. The maternal galleries radiate outward from the central nuptial chamber (formed by the male) creating a distinctive star-shaped pattern. Eggs are laid on both sides of the maternal galleries. Larval galleries develop shortly in the phloem as *I. acuminatus* is a phloemycetophagous species associated with *Ophiostoma brunneo-ciliatum* and *Ambrosiella macrospora*. Before emerging, callow adults need a period of maturation feeding that takes place in the phloem close to the galleries where the larvae have developed. The adults infest the upper part of the trunk and the branches, as a bark thickness of 2-3 mm is preferred. Literature reports *I. acuminatus* as monovoltine in Scandinavia and bivoltine in southern Europe, overwintering as adult mainly under the bark of the infested trees. However, overwintering in the litter has been considered possible in northern populations. Spring emergence begins slowly when air temperature reaches 14°-16°C, but the real mass flight occurs when temperature exceeds 18°C. As many other bark beetle species, *I. acuminatus* have a very pronounced spatial patterning linked to the cooperative attack strategy it adopts, resulting in a ‘spot infestation’.

This dissertation addresses poorly known aspects of the biology of the bark beetle observed during the outbreak that started in 2005 in the south-eastern Alps, with particular focus on:

- voltinism, phenology and breeding performance in relation to both temperature and colonisation density, in order to determine how these factors may affect insect performance and population dynamics in a climate-change perspective;
- spatial-temporal dynamics of the infestations during the different phases of the outbreak, in order to model bark beetle activity and behaviour;
- effects of a sanitation felling on spot dynamics and damage level, in order to help management decisions making about whether to apply active control measures;
- natural enemy complex associated with *I. acuminatus*, in order to assess the role of parasitoids and predators on population dynamics.

The results described in chapters II-V of this thesis have been incorporated in four distinct manuscripts, one of them currently under review and the others in preparation. The first chapter (Introduction) contains an overview of the work and preliminary results, which have already been published in Forest@ (2010, vol. 7: 259-267 pp).

Introduction

Gravi infestazioni di bostrico acuminato in formazioni alpine di pino silvestre

Published as:

Faccoli M, Colombari F, Dal Pont C, Finozzi V, D'Ambros E, Battisti A, 2010.
Gravi infestazioni di bostrico acuminato in formazioni alpine di pino silvestre.
Forest@ 7: 259-267. - [online: 2010-12-02] URL: http://www.sisef.it/forest@/.

I collected and analyzed most of the data and contributed to the writing of the manuscript.

doi: 10.3832/efor0644-007

Gravi infestazioni di bostrico acuminato in formazioni alpine di pino silvestre

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Abstract: *Large outbreaks of Ips acuminatus in Scots pine stands of the Italian Alps*. In the last years, many Scots pine (*Pinus sylvestris*) stands have been severely attacked by the bark beetle *Ips acuminatus* (Coleoptera Curculionidae Scolytinae). In the outbreak area of San Vito di Cadore (Eastern Dolomites), the number of attacked trees since 2005 and both the emergence of bark beetles and natural enemies have been assessed. The investigated forests showed dozens of easily recognizable infestation spots with size ranging from about 20-30 trees (small spots) up to 300 trees (large spots). These infested spots evolved quickly, while new ones appeared within a radius of few hundreds of meters. During the last 5 years (2006-2010) we sampled branches from small and large spots and lodged them into emergence cages: adults of *I. acuminatus* as well as natural enemies were collected weekly, identified and counted. At the same time, a monitoring program of the surveyed pine stands was carried out to check the enlargement of old spots and the appearance of new ones. Voltinism and phenology of *I. acuminatus* were investigated by pheromone traps baited with different lures (Austrian *vs*. Spanish lures). The effects of a sanitation felling of about 4500 infested trees, carried out by the Regional Forest Service in autumn 2007 on *I. acuminatus* population were also assessed. Throughout the whole sampling area *I. acuminatus* resulted bivoltine, with the highest density attained during the first generation. However, a part of the population still evidenced a monovoltine behaviour. The realized sanitation felling strongly reduced both breeding sites and the number of infested trees observed during the following year. Moreover the pheromone-baited traps gave useful information about changes in bark beetle population density; the trapping efficiency of Spanish lure resulted clearly higher than the Austrian one. Finally, the recorded parasitism may have a role in outbreak dynamics as it was significantly higher during the second host generation, in both small and large spots.

Keywords: *Ips acuminatus*, Scots pine, Pine decline, Bark beetles, Mass-trapping, Sanitation felling

Received: Sep 02, 2010; Accepted: Nov 05, 2010

Citation: Faccoli M, Colombari F, Dal Pont C, Finozzi V, D'Ambros E, Battisti A, 2010. Gravi infestazioni di bostrico acuminato in formazioni alpine di pino silvestre. Forest@ 7: 259-267. - [online: 2010-12-02] URL: http://www.sisef.it/forest@/.

Il deperimento del pino silvestre sulle Alpi

Negli ultimi anni le formazioni di pino silvestre (*Pinus sylvestris*) hanno subito severi fenomeni di deperimento e mortalità che hanno interessato vasti settori continentali delle Alpi Italiane (Valle d'Aosta, Piemonte, Alto Adige, Lombardia, Veneto), Austriache (Tirolo, Carinzia, Stiria), Svizzere (Vallese) e Francesi (Savoia e Provenza orientale - Forster & Zuber 2001, Dobbertin et al. 2007, AA.VV. 2008). I primi segnali del declino della vitalità del pino silvestre comparvero nell'Europa centro-meridionale già a partire dagli anni '80 del secolo scorso (Vallet 1981), in seguito agli alti livelli di inquinamento atmosferico e di deposizioni acide misurati in quegli anni. L'origine chimica del deperimento fu tuttavia presto esclusa in seguito al manifestarsi di nuovi fenomeni di mortalità avviati a partire dagli anni '90, concentrati principalmente a quote inferiori ai 1200 m. Una recente analisi dello stato di salute delle pinete delle Alpi occidentali (Forster & Zuber 2001, Dobbertin et al. 2007, AA.VV. 2008) ha rilevato un deperimento da medio a grave in molte delle situazioni esaminate:

i dati evidenziano la recessione del pino silvestre nelle foreste di media e bassa quota e la presenza di estese zone di moria distribuite in modo apparentemente casuale sia sui versanti settentrionali sia su quelli meridionali, sebbene le pinete di alta quota siano relativamente prive di danni riconducibili al deperimento. É interessante notare che i versanti a maggiore pendenza esibiscono i tassi di mortalità più alti, che in alcuni casi raggiungono il 35% dell'area basimetrica del comprensorio forestale, un valore dieci volte superiore alla mortalità ordinaria (AA.VV. 2008).

Il deperimento del pino silvestre si manifesta con un quadro sintomatologico complesso, variabile e generalmente aspecifico. Improvvisi ingiallimenti della chioma e disseccamento dei rami e del cimale sono osservabili nel periodo estivo, associati ad una progressiva e precoce filloptosi degli aghi più vecchi. Nei casi più gravi l'evento culmina con la morte delle piante, che si può manifestare o in modo improvviso, durante la primavera, o dopo un deperimento lungo e progressivo, in estate. Il fenomeno può riguardare sia singoli individui, sia gruppi di piccole o medie dimensioni. In molti casi ai sintomi aspecifici si sovrappongono quelli dovuti alla presenza di particolari agenti parassitari o patogeni, definiti di debolezza, che colonizzano piante in precarie condizioni vegetative (Forster & Zuber 2001, Dobbertin et al. 2007).

Le cause di questo deperimento sono tuttora oggetto di numerose ricerche, sebbene appaia sempre più evidente l'azione congiunta di molteplici fattori che includono le variazioni climatiche, con prolungati deficit idrici, l'abbandono della gestione selvicolturale di pinete montane, con l'avvio di successioni forestali a favore di latifoglie xerofile, e la comparsa di infezioni fungine e infestazioni di insetti parassiti del pino silvestre.

Gli scolitidi dei pini

Nell'ambito degli insetti fitofagi di interesse forestale, le specie che si sviluppano a spese delle parti durevoli delle piante (xilofagi *sensu lato*) rappresentano la componente più incisiva per la sopravvivenza dell'ospite vegetale attaccato e, nel lungo termine per il mantenimento e la funzionalità di interi soprassuoli. Gli effetti immediati delle loro pullulazioni si registrano soprattutto in popolamenti sofferenti per scompensi di natura climatica. La presenza di questo gruppo funzionale è infatti spesso intimamente correlata all'azione di deperimenti che riducono il vigore generale dell'ospite (Cedervind et al. 2003). Tra le entità xilofaghe strettamente legate al pino silvestre e maggiormente coinvolte nei fenomeni di mortalità di tali soprassuoli, i coleotteri scolitidi (Coleoptera Scolytidae), e in particolare quelli afferenti ai generi *Tomicus* (*T. minor* e *T. piniperda*) e *Ips* (*I. acuminatus* e *I. sexdentatus*), rivestono indubbiamente un ruolo di primo piano con infestazioni di intensità e frequenza crescenti in molti ambienti alpini italiani (Faccoli et al. 2005, Colombari et al. 2008) ed europei (Vallet 1981, Nierhaus-Wunderwald & Forster 2000, Cedervind et al. 2003, Dobbertin et al. 2007). Il riscaldamento climatico in atto altera le con-

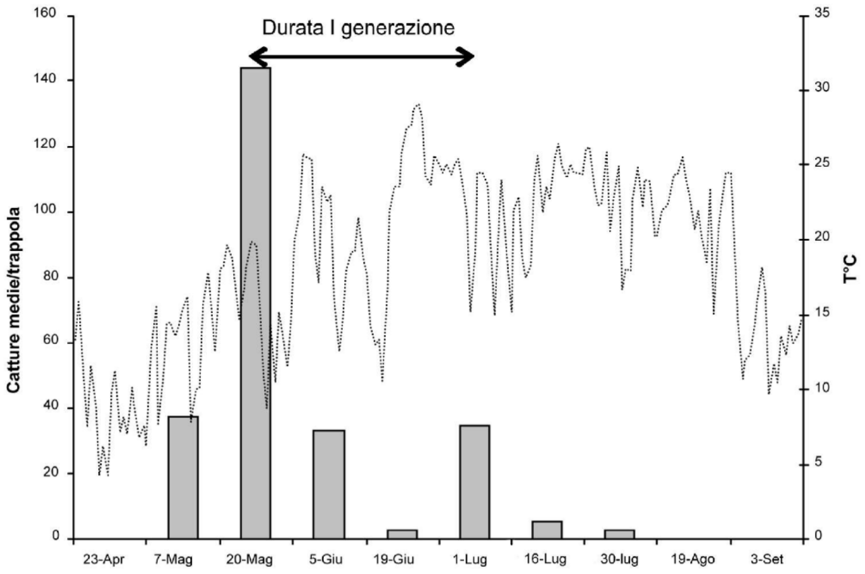

Fig. 1 - Catture medie di *Ips acuminatus* avute nella primavera-estate 2008 in Cadore e andamento delle temperature massime giornaliere.

dizioni vegetative di molte pinete, indebolite sempre più da gravi fenomeni di aridità estiva ed invernale che influenzano la suscettibilità di tali popolamenti ad attacchi di insetti xilofagi.

Il bostrico acuminato

Il coleottero scolitide *Ips acuminatus* (Gyllenhal), comunemente noto come bostrico acuminato, è fra i più comuni agenti parassitari che approfittano del deperimento delle pinete di pino silvestre. Negli ultimi decenni si sono infatti verificate gravi pullulazioni in diversi paesi europei collocati al margine meridionale dell'areale di distribuzione del pino silvestre, quali Francia (Vallet 1981, Herard & Mercadier 1996, Legrand 1996), Spagna (Romanyk 1977) e Svizzera (Forster & Zuber 2001, Dobbertin et al. 2007). In Italia sono stati documentati attacchi piuttosto consistenti in Trentino e Alto Adige a partire dal 1995 (Salvadori & Ambrosi 1997), Valtellina dal 2001 (Lozzia & Rigamonti 2002), e Calabria dai primi anni 2000 a carico del pino loricato, in associazione con altri scolitidi (Frisullo et al. 2003, Tranfaglia et al. 2004). Dal 2005 anche numerose pinete della provincia di Belluno, e in particolare del Cadore, manifestano ingenti danni (Faccoli et al. 2007, Colombari et al. 2008, Colombari et al. 2009a, Colombari et al. 2009b, Colombari et al. 2009c, Colombari et al. 2009d).

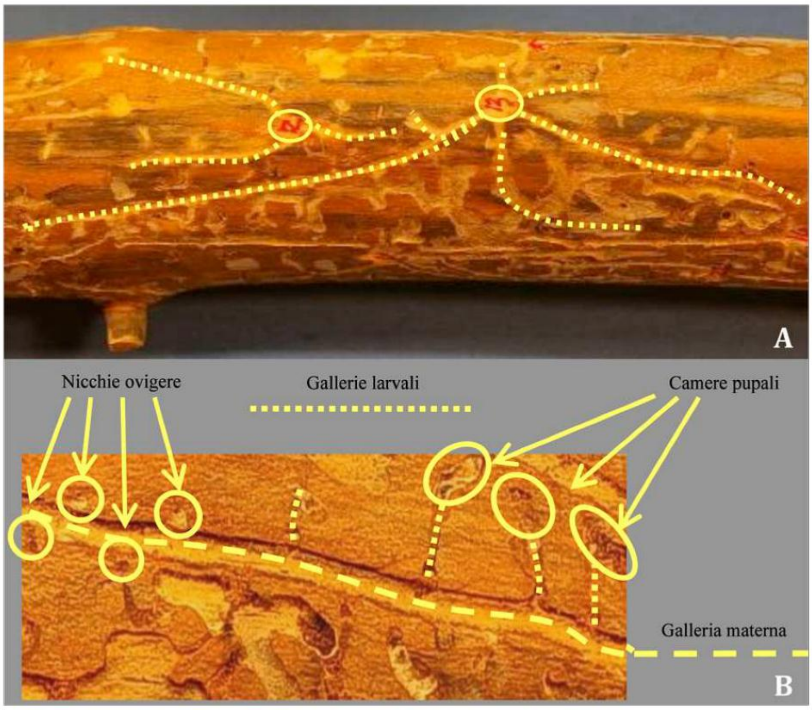

Fig. 2 - Sistemi riproduttivi sottocorticali di *Ips acuminatus*. (A): sono chiaramente visibili i vestiboli (cerchiati in giallo) e le numerose gallerie materne che si originano da essi (evidenziate dal tratteggio); (B): nicchie ovigere, gallerie larvali e celle pupali (foto: F. Colombari).

Date le sue piccole dimensioni (2.2 - 3.5 mm) il bostrico acuminato colonizza la corteccia della parte medio-alta del fusto e pressoché tutte le branche principali. In primavera gli adulti volano alla ricerca delle piante ospiti e il picco delle catture si registra con temperature massime dell'aria almeno pari o superiori a 16° C (Fig. 1). I primi a giungere sulla corteccia sono i maschi che, dopo aver scavato una "camera di accoppiamento" o "vestibolo", con una miscela di feromoni di aggregazione attraggono da 2 a 7 femmine. In seguito all'accoppiamento ciascuna femmina scava sotto corteccia una "galleria materna" che, a seconda della densità di colonizzazione, può raggiungere gli 11 cm di lunghezza, presentando un andamento parallelo alle fibre del legno (Fig. 2A). Lungo i due lati della galleria, entro piccoli loculi detti "nicchie ovigere", vengono singolarmente deposte le uova (Fig. 2B), il cui numero dipende dalla lunghezza della galleria essendo la distanza tra le nicchie piuttosto costante (circa 0.6 cm). Le larve neonate iniziano a nutrirsi del floema scavando, perpendicolarmente alla galleria materna, brevi "gallerie larvali" ingombre di rosura e con diametro progressivamente crescente (Fig. 2B). Al termine dello sviluppo le larve mature affrontano la metamorfosi all'interno di "celle pupali" che spesso vanno a intaccare l'alburno (Fig. 2B). Il neo-adulto sfarfallato, per completare lo sviluppo e raggiungere la maturità sessuale, trascorre un ulteriore periodo sotto corteccia scavando gallerie di nutrizione ad andamento irregolare, sovente sovrapposte al sistema di gallerie sopra descritto. Lo sviluppo completo richiede circa 6 settimane (Fig. 1) e verso la fine di giugno compaiono i nuovi adulti. La generazione estiva, ovvero la seconda, produrrà individui che sverne-

Fig. 3 - Nucleo di piante attaccate con chiome arrossate in seguito all'attacco del bostrico acuminato (foto: V. Finozzi).

ranno sottocorteccia allo stadio adulto.

Gli attacchi conducono rapidamente a morte le piante, che nel giro di poche settimane presentano chiome rosseggianti a seguito del disseccamento degli aghi (Fig. 3). Inoltre, sebbene le gallerie materne e larvali incidano il legno solo superficialmente, il materiale infestato subisce un danno tecnologico dovuto all'invasione dell'alburno da parte di funghi simbionti agenti di azzurramento, sempre rilevati nel materiale abbattuto (Fig. 4). *I. acuminatus* è infatti una specie fleomicetofaga, regolarmente associata con tre principali specie fungine: *Ophiostoma brunneo-ciliatum*, *O. ips* e *Ambrosiella macrospora*. Le prime due specie sono patogene e interagiscono con le difese della pianta limitando la possibilità di questa di difendersi dagli attacchi dello scolitide (Lieutier et al. 1991). *A. macrospora* è invece un fungo dell'ambrosia, fondamentale per l'alimentazione delle larve ma non patogeno per la pianta (Francke-Grosmann 1952).

Fig. 4 - Azzurramento dell'alburno dovuto a funghi del genere *Ophiostoma* inoculati dagli adulti di *Ips acuminatus* (foto: F. Colombari).

Fig. 5 - Andamento temporale delle catture di *Ips acuminatus* e ammontare dei danni provocati nei popolamenti di pino silvestre monitorati.

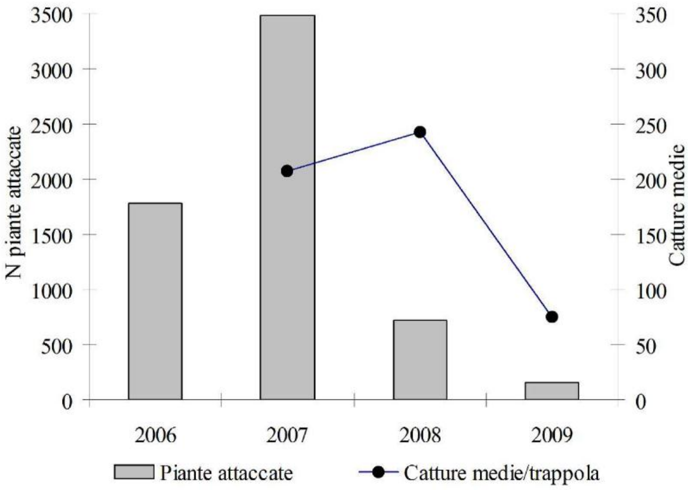

Fig. 6 - Trappola a feromoni del tipo "ad imbuto" impiegata in Cadore per il monitoraggio delle popolazioni di *Ips acuminatus* (foto: F. Colombari).

Misure di monitoraggio e controllo

Nell'ambito di un'ampia indagine relativa all'analisi di vari aspetti della bio-ecologia e del controllo di *I. acuminatus*, numerose popolazioni di *I. acuminatus* distribuite lungo l'intero arco alpino - Valle d'Aosta, Valtellina (SO), Valcamonica (BS), Val Venosta (BZ), Cadore (BL) e Val Dogna (UD) - sono state ripetutamente campionate al fine di poter analizzare le variazioni temporali e geografiche dei principali parametri presi in considerazione. In particolare, le infestazioni comparse in provincia di Belluno sono state studiate con attenzione già a partire dal 2006. I primi risultati mostrano come gli alberi attaccati dal 2006 al 2010, oltre 6000 piante, si presentino ripartiti in decine di nuclei di dimensioni variabili (da un minimo di 2-3 a oltre 150 piante l'uno), successivamente censiti, cartografati e campionati misurandone inoltre i principali parametri selvicolturali e fitosanitari. L'analisi dei dati mostra che le aree colpite si distribuiscono in modo apparentemente casuale sul territorio e che non si espandono nel tempo, ma nelle loro vicinanze - anno dopo anno - compaiono nuove aree che seguiranno la stessa evoluzione (Faccoli et al. 2007). Il 2006 e 2007 hanno presentato elevate densità di infestazione con danni particolarmente gravi (Fig. 5). Nell'autunno del 2007 si è quindi intervenuti con un taglio fitosanitario finalizzato all'abbattimento e rimozione di tutte le piante colpite nel corso dei due anni precedenti. L'azione è stata condotta mediante esbosco con elicottero e distruzione per cippatura di oltre 4500 piante distribuite in 47 nuclei di infestazione. Il taglio ha interessato anche alcune piante sane presenti all'interno o lungo i margini dei nuclei di infestazione. L'intervento si è di-

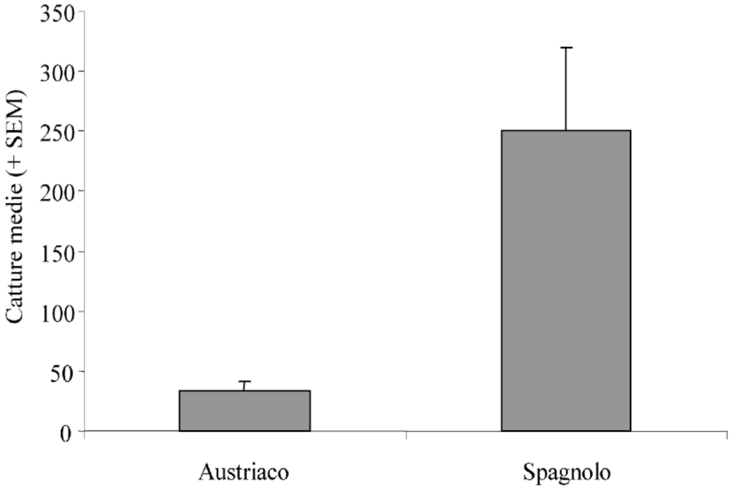

Fig. 7 - Differenze nelle catture medie degli adulti di bostrico acuminato in trappole innescate con feromoni di produzione austriaca e spagnola.

mostrato estremamente positivo in quanto ha ridotto di quasi l'80% il danno registrato nell'anno seguente (Fig. 5), e ha avviato alla conclusione una pullulazione che durava da almeno 3 anni.

Oltre al censimento dei danni, a partire dal 2007 è stata intrapresa una sorveglianza delle popolazioni di *I. acuminatus* mediante trappole "ad imbuto" (Fig. 6) innescate con uno specifico feromone di aggregazione di produzione austriaca (AcuWit®, Witasek®). Le trappole sono state collocate nei vari nuclei d'infestazione in numero variabile in relazione alle dimensioni del nucleo, mantenendo tuttavia una densità costante pari a circa 1 trappola ogni 20 piante colpite. Il controllo delle catture e lo svuotamento delle trappole è stato eseguito ogni 15 giorni a partire da metà aprile fino a metà settembre. A metà giugno, dopo due mesi dall'inizio delle prove, gli erogatori dei feromoni sono stati sostituiti. I periodi di volo e il numero di generazioni riscontrate nei boschi monitorati sono risultati essere molto simili da un anno all'altro, fatto salvo qualche piccola variazione dovuta alle locali condizioni climatiche. L'insetto inizia l'attività primaverile a fine aprile (Fig. 1); dopo circa un mese (fine maggio) si hanno in genere deboli catture di insetti riemergenti alla ricerca di nuovi ospiti su cui avviare "generazioni sorelle" (Fig. 1). Nel corso dell'estate le catture sono generalmente molto scarse e l'assenza di nuovi consistenti sfarfallamenti indica che la specie affronta l'inverno con individui di seconda generazione rimasti all'interno dei pini attaccati in giugno. Benché le catture medie siano molto basse (Fig. 5), il loro andamento temporale riflette in parte quello dei danni, con valori elevati nel corso del 2007 e 2008, e poi sensibilmente inferiori nel 2009 (-70%), a indicare una popolazione in declino (Fig. 5). L'uso di tali trappole sembra quindi essere un valido strumento per il monitoraggio delle popolazioni, ma non per il loro controllo, data la ridotta capacità attrattiva nei confronti degli adulti. Per cercare di ovviare a questo problema, e per poter disporre di nuove tecniche di controllo, nel 2009 è stato saggiato un formulato sperimentale di un nuovo feromone di aggregazione di produzione spagnola (SEDQ®). La prova è consistita nel confrontare l'efficacia attrattiva dei due feromoni (austriaco e spagnolo), innescando 20 trappole per ciascun formulato. La miscela spagnola si è dimostrata estremamente più attrattiva con un'efficacia circa 7.5 volte superiore a quella del feromone austriaco (Fig. 7), con densità medie di cattura di 250 contro 34 insetti per trappola. La migliore efficacia dei nuovi formulati sperimentali prospetta un possibile impiego dei feromoni non solo nel monitoraggio, ma anche in programmi di controllo basati sulle catture massali, come già avviene in altre specie di scolitidi (Faccoli & Stergulc 2008).

Inoltre, a partire dal 2007 le popolazioni di *I. acuminatus* sono state monitorate anche mediante raccolta di rami attaccati contenenti stadi di sviluppo dello scolitide. I tronchetti sono stati collocati entro fotoeclettori mantenuti a temperatura ambiente in attesa del completo sviluppo degli insetti e del loro sfarfallamento. I neo-adulti emergenti sono stati raccolti e contati al fine di definire la curva di volo del bostrico acuminato e di eventuali altre specie presenti, nonché la densità dell'insetto per dm^2 di corteccia. Le epoche di sfarfallamento e l'entità dei picchi di volo di *I. acuminatus* sfarfallati dai tronchetti in allevamento corrispondono ai picchi di cattura riscontrati in campo con l'ausilio delle trappole. La maggior parte degli individui sfarfallati si riscontra infatti ad inizio primavera, con un contingente pari a circa 6 individui per dm^2 di corteccia infestata nel corso dell'anno precedente, mentre la prole della prima generazione emerge in giugno con un numero molto limitato, pari a meno di un individuo per dm^2. Tale ridotto numero sembra dovuto al comportamento monovoltino di una parte della popolazione, che compirebbe un solo ciclo riproduttivo. Lo scortecciamento dei tronchetti colonizzati in primavera ha permesso di appurare che una parte degli insetti di prima generazione rimane effettivamente sottocorteccia fino all'anno successivo senza avviare una seconda generazione.

Prospettive future

Ips acuminatus è una specie comune nelle pinete alpine, ma per molti anni considerata di scarso interesse selvicolturale. Di norma infatti è un insetto secondario in grado di colonizzare alberi la cui capacità di difesa risulta gravemente compromessa da eventi occasionali di origine abiotica (siccità, elevate temperature, incendi, schianti da vento, neve o valanghe) o biotica (forti defogliazioni, intense infezioni fungine - Chararas 1962). In questi casi lo xilofago, presente a bassi livelli di popolazione, aumenta rapidamente la propria consistenza numerica dando luogo a pullulazioni spesso aggravate dall'intervento di altre specie. Le elevate densità di popolazione raggiunte consentirebbero poi all'insetto di comportarsi da fitofago primario in grado di colonizzare anche piante apparentemente vigorose.

Nella maggior parte dei casi le infestazioni di *I. acuminatus* segnalate sul territorio nazionale interessano pinete con prevalente funzione protettiva e paesaggistica; si tratta infatti di boschi situati in aree ad alta frequentazione turistica estiva e invernale. La loro presenza costituisce dunque un elemento insostituibile del paesaggio alpino, che può venire gravemente compromesso dalla presenza di nuclei di piante morte in piedi visibili anche a chilometri di distanza. Tali nuclei sono riconoscibili come macchie dapprima rosse e poi grigie sparse nel manto boschivo di formazioni sempreverdi. Inoltre le pinete di pino silvestre rappresentano spesso formazioni pioniere edificate su ghiaioni di sfaldamento di rocce carbonatiche, ambienti difficili e instabili che non consentono un'agevole evoluzione di suoli e soprassuoli e che sarebbero difficilmente occupabili da altre specie arboree. Si tratta di colonizzazioni spontanee di pendii franosi lasciate crescere senza cure particolari, e che ora si trovano frequentemente esposte ad avversità di natura parassitaria indotte dalle difficili condizioni stazionali e dalla compromessa vigoria degli alberi. In tale sfavorevole situazione vegetativa, gli ormai noti cambiamenti climatici - fra cui il variato ritmo delle precipitazioni, la sempre più breve e sottile copertura nevosa e le crescenti temperature estive (Solomon et al. 2007) - possono contribuire ad innescare gravi processi di deperimento. Le recenti anomalie climatiche sembrano infatti avere un ruolo determinante negli equilibri fra piante ospiti e fitofagi (Rouault et al. 2006), indebolendo i primi a vantaggio dei secondi o influenzandone profondamente la diffusione, come osservato nel caso di altri parassiti forestali (Battisti et al. 2005, Battisti et al. 2006).

Il contenimento di tali infestazioni può basarsi su tecniche selvicolturali finalizzate al mantenimento delle pinete in buone condizioni vegetative, o sulla progressiva sostituzione di pinete presenti in realtà climatiche ormai non più idonee a soddisfare le esigenze del pino silvestre. In queste situazioni il costante monitoraggio delle popolazioni di *I. acuminatus*, basato sia sull'impiego di trappole a feromoni che sul censimento dei nuclei d'infestazione, risulta essere un primo fondamentale strumento di supporto decisionale. In considerazione degli elevati costi di realizzo, tagli fitosanitari finalizzati alla rimozione e distruzione del materiale infestato possono trovare giustificazione solo nel caso di rischi di gravi pullulazioni o in formazioni di particolare valore economico, ecologico o paesaggistico, come avvenuto nelle coniferete del Cadore. Ulteriori studi sono tuttora in corso per chiarire le strette relazioni fra pianta ospite, condizioni stazionali, insetto fitofago e organismi ad esso associati in un contesto di protezione delle pinete alpine.

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Chapter I

Life history traits promoting outbreaks of the pine bark beetle Ips acuminatus *(Coleoptera: Curculionidae, Scolytinae) under climate change*

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Submitted to ***European Journal of Forest Research*** as:

Colombari F., Battisti A., Schroeder L.M., Faccoli M.
Life history traits promoting outbreaks of the pine bark beetle Ips acuminatus
(Coleoptera: Curculionidae, Scolytinae) under climate change

I collected most of the data, analysed them and drafted the manuscript.

Abstract

The pine bark beetle *Ips acuminatus* has recently increased frequency and intensity of outbreaks in *Pinus sylvestris* stands in the Alps. During a three-year period, we investigated life history traits of the species that may have adaptive value in response to climate change. In the south-eastern Alps, *I. acuminatus* becomes active in early spring when the air temperature reaches 14°C, suggesting the presence of a local population adapted to low temperature. Such an early emergence allows the complete development of a second generation, even if only a portion of the population is truly bivoltine. As a consequence, there are two main attack periods, the first in early spring and the second in summer, resulting in different trees being colonised each time. Irrespective of the generation, a large part of the beetles leaves the breeding substrate before hibernation, and this is interpreted as an escape from natural enemies associated with the bark. These findings suggest that the populations of *I. acuminatus* of the south-eastern Alps may take benefit from climate change as they have more opportunities to complete the second generation and to escape from mortality factors associated with a long permanence in the bark. In addition, the extended period of tree colonisation offers more possibilities to locate suitable hosts and to build up outbreak densities.

Keywords: pine bark beetle, voltinism, colonisation density, mortality, temperature.

Introduction

Many insect populations, including bark beetles, are known to respond to the recent climate change (Dobbertin et al. 2005, 2007; Berg et al. 2006; Bigler et al. 2006; Lange et al. 2006; Jönsson et al. 2007; Faccoli 2009), promoting a progressive forest decline especially in the alpine regions. In the last years, high temperature coinciding with periods of drought has resulted in a global-change-type drought (Breshears et al. 2005), which reduces developmental time, increases voltinism and the infestation pressure of bark beetles (Wermelinger and Seifert 1998).

Outbreaks of the pine engraver beetle *Ips acuminatus* (Gyllenhal) (Coleoptera: Curculionidae, Scolytinae) have been recently reported in many Scots pine forests in the western (Lozzia and Rigamonti 2002; Wermelinger et al. 2008) and eastern Alps (Dolomites, NE Italy) (Colombari et al. 2008). For many years *I. acuminatus* has been reported as a species of minor economic importance (Bakke 1968), causing damage mainly by transmitting blue stain fungi to saw-logs. But in the last decades it has been included among the ten most damaging wood boring insect in Europe as a result of the increasing tree mortality caused by this species (Grégoire and Evans 2004). As low temperature is known to be a limiting factor for *I. acuminatus* flight activities and brood development (Bakke 1968), it is likely that climate change may aggravate the threat to pine forest health, either with the beetle alone or in combination with secondary pathogens (Rigling et al. 2000; Rebetez and Dobbertin 2004). This could explain the increasing importance of *I. acuminatus* in the Scots pine decline recently observed in the Alps (Wermelinger et al. 2008).

The life history of *I. acuminatus* varies according to study sites. Literature reports *I. acuminatus* as monovoltine in Scandinavia (Bakke 1968) and bivoltine in southern Europe (Chararas 1962; Hernandez et al. 2004), although this information is rather fragmentary and does not concern alpine populations. Overwintering occurs as an adult mainly under the bark of the infested trees (Bakke 1968; Lekander et al. 1977; Hernandez et al. 2004; Wermelinger et al. 2008), but overwintering in the litter has been considered possible (Francke-Grosmann 1963; Lekander et al. 1977) in northern populations. Spring emergence begins slowly when air temperature reaches 14°-16°C (Bakke 1968), but the real mass flight occurs when temperature exceeds 18°C (Bakke 1968; Lekander et al. 1977;

Hernández et al. 2004, 2007). The above mentioned aspects are still unclear for the alpine populations and need to be clarified. In addition, as far as we know, no earlier studies were conducted on mating systems and competition of *I. acuminatus* on living trees under natural conditions or on the relationships between colonisation densities and brood performance over generations and years.

During an outbreak of *I. acuminatus* that has spread in the south-eastern Alps since 2006, we investigated voltinism, phenology and breeding performance in relation to both temperature and colonisation density over a three-year period, in order to determine how these factors may affect insect performance and population dynamics in a climate change perspective.

Materials and methods

Organism and study area

Ips acuminatus is a polygamous species with up to 6-12 females joining each male within the gallery systems. The egg galleries (excavated by females) radiate outward from the central nuptial chamber (formed by the male) creating a distinctive star-shaped pattern (Kirkendall 1989). Eggs are laid on both sides of the egg galleries. Larval galleries develop shortly in the phloem as *I. acuminatus* is a phloemycetophagous species associated with *Ophiostoma brunneo-ciliatum* and *Ambrosiella macrospora* (Mathiesen 1950; Bakke 1968; Francke-Grosmann 1952, 1963; Lieutier et al. 1991; Kirisits 2004). Before emerging, callow adults need a period of maturation feeding that takes place in the phloem close to the galleries where the larvae have developed. The adults infest the upper part of the trunk and the branches, as a bark thickness of 2-3 mm is preferred (Bakke 1968). A specific trait of this species is the coexistence of both sexual and parthenogenetic reproduction with pseudogamous females (Løyning and Kirkendall 1996; Løyning 2000; Meirmans et al. 2006).

The study area, with *I. acuminatus* infested Scots pine stands, is located in the Cadore Valley (46°27' N, 12°12' E, 900-1,300 m) in northern Italy, on dolomite and limestone bedrock covered by shallow soils on south-western facing slopes. The stands have a mean density of about 300 stems per hectare and are composed by mature trees (>100 years) with a poor annual growth because of nutrient and water limitations. Main functions of these stands are reducing soil erosion and improving the scenic value of the landscape, as the valley is one of the major tourist attractions in the Alps. Since 2006, the *I. acuminatus* outbreak has killed thousands of pines in the valley. The killed trees are clumped in multiple-tree spots, very different in size and distributed over an area of about 2,000 ha. Rainfall and temperature data of the study area were collected from a weather station of the Padova University located inside the outbreak area at an altitude of 1,100 m a.s.l.

Population survey

Phenology and voltinism of *I. acuminatus* were monitored by multi-funnel traps (Witasek®) set in active spots of *I. acuminatus* killed trees (colonised in the previous year) in early spring 2007 (36 traps), 2008 (9 traps) and 2009 (5 traps). Traps were baited with a species-specific pheromone lure (Acuwit®, Witasek) and checked daily from mid-March (2007) or early April (2008 and 2009) to assess the timing of the initiation of *I. acuminatus* flight; then the traps were emptied twice a month until no beetles were caught. The pheromone dispensers were replaced once in June, except in 2009 when the trapping covered only the spring flight period. All trapped adults of *I. acuminatus* were counted and sexed.

Colonisation and breeding performance of *I. acuminatus* was studied in 21 randomly selected spots with killed trees. The sampling was conducted in April and June in 2007 and 2008 (N = 6 in April 2007, June 2007 and April 2008; N = 3 in June 2008). The spots selected in April resulted from trees attacked in the summer of the previous year, whereas those selected in June consisted of trees colonised in the spring of the current year. Three colonised trees were felled in each spot, summing up to a total of 63 trees. For each one of these trees diameter at breast height, total tree height, height and diameter at the beginning and at the end of the *I. acuminatus* colonisation points were recorded. The Relative Infested Bole Height (RIBH) was computed for all felled trees as: (infested bole height)/(total tree height) (Fargo et al. 1979). In addition in 2007, on one tree per spot (summing up to thirteen trees, because in addition to the twelve trees an isolated infested pine was cut), the Total Colonised Bark Surface (TCBS) was calculated as the sum of the Infested Bole Area (IBA) and the Infested Branch Area (IBRA). IBA was computed as:

IBA = diameter x length of the attacked trunk portion, where the diameter value was calculated as the mean of the trunk diameters at the beginning and end of the insect colonisation points.

IBRA of each n-whorl was computed as:

$IBRA_n$ = (diameter x length of the attacked portion of one branch per whorl) x number (n) of colonised branches in the whorl, where the diameter value was calculated as the mean of the branch diameters at the beginning and end of the insect colonisation.

Hence, the TCBS was equal to: $TCBS = IBA + \sum^{1}{}_{n} IBRA_{n.}$

From each one of the 63 felled trees, ten colonised branch samples (approximately 45 cm long and 5 cm in diameter) were randomly selected. The branch samples were cut and transported to the campus of the Agriculture Faculty of the Padova University in the same day. The ten branch samples from each tree were caged together in plastic pipe-cages (diameter 40 cm, length 50 cm) closed at both ends by a fine wire mesh and placed under outdoor conditions. Temperature was recorded on site. Cages were checked daily and all emerging *I. acuminatus* adults were counted and sexed, while the associated species were kept for further analysis.

At the end of the *I. acuminatus* emergence from the cages, branches were debarked and all dead or alive *I. acuminatus* recorded. On one randomly chosen branch per cage (i.e. per tree), all mating systems were analysed in order to estimate the density of colonising males (*i.e.*, number of nuptial chambers), females (*i.e.*, number of egg galleries) and, consequently, total colonisation density (i.e., number of males and females). Furthermore, for one mating system per branch the length of egg galleries was measured, and the number of egg niches, larval galleries, pupal cells and galleries resulting from maturation feeding by the new generation adults was recorded. It was assumed that each egg-niche had contained one egg. The number of young larvae was estimated from the number of larval galleries starting from the egg-niches, whereas the number of mature larvae was estimated from the larval galleries ending with a slight enlargement in diameter. The number of pupal cells was considered as number of pupae and the number of pre-emerging adults was estimated from the number of irregular galleries of maturation feeding starting from the pupal cells. Lastly, the percentage of beetles that have emerged to hibernate outside the breeding material was assessed considering the number of maturation feedings on the branches and the number of emerging adults from the branches. Only on branches collected in 2007 (36 trees, 12 spots) were also measured size of nuptial chambers, initial egg-free part of the maternal galleries and distance between eggs along the galleries.

Statistical analysis

The thermal sums needed for the beginning and peak of spring flight (calculated for the period from January 1st to the event considered) and for brood development (calculated for the period between the observed flight peaks in 2007 and 2008) were obtained testing a set of threshold temperatures ranging between 0 and 10°C; the temperature that minimized the standard deviation of the mean thermal sum calculated over the three years was chosen as threshold temperature.

Values of all variables recorded from each tree were averaged within the spot (N = 21). All mean values are reported ± standard deviation (SD), unless otherwise specified. Two independent factors, generation and year, and their interactions, were tested by a two-way ANOVA for each variable (density of parent beetles and preimaginal stages, percentage of mortality). To fit the assumptions of the ANOVA, density and proportion data were log and arcsin-square root transformed when needed. Significance was determined at $p<0.05$.

Statistical analyses were performed using the software programs R (version 2.7.0) and Statistica 8 (Statsoft, Inc 1993).

Results

Phenology and voltinism

The first adults of *I. acuminatus* were caught after daily maximum air temperatures reached 14°C (April 5th in 2007, 23rd in 2008 and 12th in 2009). In both 2007 and 2008 trap captures showed two distinct peaks, one in spring and one in summer (Fig. 1). The first peak occurred 17, 27 and 31 days after the beginning of flights in 2007, 2008 and 2009, respectively (mean of 25 ± 7.2). Spring flight started at a thermal sum of 15.2 ± 1.6 DD (Degree Days), calculated from January 1st above the threshold of 6°C, whereas flight peak occurred at a thermal sum of 98.5 ± 2.2 DD (Tab. 1).

Considering mean trap catches of the entire flight period, beetles swarmed in summer corresponded to 19.6% and 14.0% of those swarmed in spring of 2007 and 2008, respectively. Spring and summer peaks were separated by 67 days in 2007 and 42 days in 2008. The thermal sum between the two peaks, calculated above the threshold of 14°C, amounted to 72.8 ± 5.2 DD. In rearing cages, the insect emergence recorded from branches collected in spring and summer had a pattern similar to that observed in the field, with two distinct peaks and a lower number of adults swarming in summer (from 5.80 to 40.1%).

The emergence data from branches collected in June and colonised in the same spring demonstrated that about half of the offspring of the first generation (50.5% and 55.5% in 2007 and 2008, respectively) did not emerge in summer. Moreover, only 15.7% and 4.8% (in 2007 and 2008, respectively) of the overwintering adults were effectively found hibernating under the bark in the following spring, as the rest had evidently left the tree before collection.

Tree infestation pattern

I. acuminatus colonisation generally occurred in the medium-upper part of the tree starting at a mean diameter of 14.1 ± 2.0 cm (N = 21). The mean RIBH was 0.5 ± 0.1 (N = 21), with a total tree height varying from 5.50 to 14.5 m (9.8 ± 1.8, N = 21). The TCBS ranged from 1.5 to 8.4 m^2 per tree and it was related significantly to tree diameter at breast height (TCBS = 0.387 x diameter at breast height − 3.55, N = 13) ($R^2 = 0.71$, $F_{(1, 11)} = 27.51$, $p<0.001$). It was thus estimated that the infested pines were colonized by 10,947 ± 3,948 beetles (N = 21), depending on their size (mean breast height diameter 20.2 ± 3.6 cm, N = 21).

Densities of males (nuptial chambers), females (egg galleries), and both males and females (colonisation density) were similar between and within years (Tab. 2), except for male density which showed a significant interaction between year and generation (Tab. 2). Nuptial chambers were always free of frass and their size (0.23 ± 0.02 cm^2, N = 12) did not vary with colonisation density. Harem size ranged from 1 to 7 (2.6 ± 0.3, N = 21) females per male. Galleries shorter than 1 cm did not contain egg niches and ranged from 0 to 4 per harem (1.8 ± 0.5, N = 21), increasing linearly with colonisation density ($R^2 = 0.36$, $F_{(1, 19)} = 10.67$, $p<0.005$). Differently, the mean number of egg galleries per mating system (harem size) and their length (4.7 ± 0.8 cm, range 1 to 11 cm, N = 21), decreased with increasing density of colonising males ($R^2 = 0.43$, $F_{(1, 19)} = 14.55$, $p<0.005$ and $R^2 = 0.23$, $F_{(1, 61)} = 18.4$, $p<0.0001$, respectively) (Fig. 2a). The number of eggs in each harem (26.67 ± 8,02 N = 21) was strongly correlated with both harem size ($p<0.05$) and egg gallery length ($p<0.01$). Female fecundity (eggs per gallery) varied from 4.2 to 16.7 (8.09 ± 2.10, N = 21) according to gallery length ($R^2 = 0.42$, $F_{(1, 19)} = 14.04$, $p<0.005$) (Fig. 2b). The distance between eggs along the gallery (0.76 cm ± 0.12, N = 12) was inversely related to the female fecundity (1.85 egg/cm ± 0.4, N =12) ($R^2 = 0.43$, $F_{(1, 10)} = 7.55$, $p<0.05$) (Fig. 2c). Lastly, the mean length (0.76 ± 0.13 cm, N = 12) of the egg-free part of the gallery was not correlated with any density variable.

Breeding performance and brood mortality

The mean breeding performance, calculated as percentage of eggs giving emerging adults, was higher in the second than in the first generation ($F_{(1, 19)} = 19.20$, $p<0.005$), with a mean of 16.45% per spot (± 8.00, N = 21). The offspring production, ranging from 0.003 to 5.55 new adults per female (1.47 ± 0.97, N = 21), was negatively correlated with colonisation density ($R^2 = 0.21$, $F_{(1, 19)} = 4.98$, $p<0.05$) (Fig. 3). The sex-ratio of the adults emerged from the sampled branches ranged from 1 to 1.45 females per male. The density of all preimaginal instars was higher in the second than in the first generation and, with the exception of the number of eggs, did not show significant variations among years (Tab. 2). Preimaginal mortality strongly reduced the number of individuals, as 47.7 ± 11.5 % (N = 21) of the offspring died before pupation (Fig. 4). Egg mortality (16.7 ± 5.4 %, N = 21) and young larvae mortality (12.2 ± 5.4 %, N = 21) were not affected by colonisation density. Mortality increased in mature larvae (29.0 ± 13.1 %, N = 21) and especially in pupae (62.5 ± 17.1 %, N = 21): values were higher in the first than in the second generation ($F_{(1, 17)} = 6.23$; $p<0.05$) and positively related to colonisation density ($R^2 = 0.25$, $F_{(1,19)} = 6.47$, $p<0.05$). Lastly, the mortality of the pre-emerging callow adults ranged from 0 to 90.9 % (22.2 ± 13.2 %, N = 21).

Discussion

The life history traits of *I. acuminatus* in the new outbreak area in the south-eastern Alps reveal a number of adaptations to local abiotic and biotic factors that may increase the frequency and intensity of outbreaks in a climate change perspective. First, the early spring emergence allows the beetles to complete a second generation, resulting in an extension of the period during which trees are challenged by colonising adults. As summer in the southern slope of the Alps is characterized by irregular drought events, trees will be thus more exposed to beetle attacks (Wermelinger et al. 2008). With increasing temperatures the proportion of new generation beetles reproducing before hibernation may increase, causing a higher risk of tree mortality. Second, a high proportion of the beetles overwinter away from the infested tree, possibly in the litter (Francke-Grossman 1963; Lekander et al. 1977), and thus escape natural enemies occurring under the bark of their breeding material especially when high temperature allows a longer period of activity of predators and parasitoids (Faccoli 2002).

Alpine populations appear to emerge earlier than other known populations in both northern and southern Europe. In Norway, *I. acuminatus* is included among the ‘late swarming bark beetles on pine’ as the earliest trap collections has been observed in mid-May (in June at the northern sites) and the mass flight takes place only when air temperature is greater than 18°C, even if few beetles can fly at a temperature of 14-16°C (Bakke 1968). The flight activity lasts for one or two months, depending on locations, and a second generation is excluded (Bakke 1968). In southern areas, such as Spain, *I. acuminatus* flight starts in May, when the maximum air temperature also exceeds 18°C (Hernández 2004; Pérez and Sierra 2006). Further, the flight period in Spain is prolonged and without a clear peak, so a second generation seems possible only when unusually warm temperatures allow beetles to breed until very late in the season (Hernández 2004; Pérez and Sierra 2006). On the contrary, in our field site the beetles swarm when temperature is 14°C only and the first generation is accomplished by the end of June. These findings lead to hypothesize the existence of local specific developmental thresholds in the studied population, regulating both the early start of the flight period and the precise synchronization in emergence; these factors allow the occurrence of a partial second generation in the year and a longer exposure of trees to beetle attack (Logan and Powell, 2003; Ayres et al. 2009). The model fits well with predictions made by Bentz et al. (2010) that temperature influences bark beetle population dynamics predominately through effects on life traits, facilitating the maintenance of adaptive developmental timing.

I. acuminatus has been hypothesized to have mono- or bivoltine populations according to latitude and altitude (Chararas 1962; Bakke 1968; Forster and Zuber 2001; Hernandez et al. 2004), but precise information is lacking and it is not clear whether the two generations may occur in the same tree. In our study we show that different host trees are attacked in spring and summer by beetles of two distinct swarming flights, as demonstrated by the appearance twice a year of new infested trees about two months after the flight peaks recorded by traps. The long period between the spring and summer emergence peaks (about 50 days) excludes the possibility that the second peak is the result of re-emerging parent beetles trying to establish a sister brood (Anderbrant 1986). The beetle population in the study area, however, is only partly bivoltine and this suggests the possible existence of a regulatory mechanism of voltinism such as a facultative diapause, already shown by Gehrken (1985) for northern populations. This regulatory mechanism could be affected by abiotic factors, *i.e.* specific microclimatic bark conditions as shown by Baier et al. (2007) for *I. typographus*, or by seasonal fluctuations in some factors that regulate individual fitness such as host-plant quality, food limitation, or natural enemies (Sota 1988).

Our results indicate that beetles hibernate both under the bark of the infested trees and outside of the breeding material. Branches sampled in early spring, before insect emergence, contained only about 10 % of the expected adults. This small number of beetles could not explain the occurrence of successful attacks in spring on a large number of trees, because attack densities known to be lethal to Scots pine are very high (Guérard et al. 2000), as it has been confirmed by our data. Wermelinger et al. (2008) also observed that the number of overwintering adults (0.35/dm^2) found during a study carried out in South-Western Switzerland (Rhone valley) was lower than expected based on the number of infested trees. The most plausible explanation is that the missing beetles emerged before the winter of the previous year. Traps did not detect this flight because beetles looking for hibernation sites – such as bark or forest litter – are probably not responding to pheromones. Although in northern Scandinavia most of the *I. acuminatus* population spend the winter under the bark of trees where they have developed (Bakke 1968), some authors (Francke-Grosmann 1963; Lekander et al. 1977) reported the possible hibernation of *I. acuminatus* adults in the forest litter. There is no precise information, however, about this behaviour and how it could affect beetle survival. *I. acuminatus* is a freezing-susceptible species, but newly emerged adults freeze at a temperature of -19°C and hibernating adults can tolerate temperature down to about -33°C (Bakke 1968; Gehrken 1983, 1985). It is thus unlikely that adults leave the tree to find less extreme conditions than those provided by pine bark (Bakke 1968; Lombardero et al. 2000). We hypothesize instead that migration to different overwintering sites can be considered as an escape from natural enemies, known to be important factors that could impact the breeding performance of *I. acuminatus* in Europe (Kenis et al. 2004) and also in the study site (unpublished data). As warmer and wetter winters are generally predicted by climate change scenarios (Rosenzweig et al. 2007), it is likely that there will be a higher activity of predators, parasitoids, and pathogens associated with beetles under the bark (Bentz et al. 2010), whereas individuals dispersed elsewhere will be less exposed.

Synchronised early colonisation and enemy escape can be important factors for the reproductive success of a species that is characterised by very high attack density in our (7.25/dm^2 resulting in 100% killed trees) as well in other studies (8.5/dm^2 resulting in 95% killed trees, Guérard et al. 2000). Increasing bark colonisation density has been associated with increasing mortality due to intraspecific competition (Beaver 1974; Kirkendall 1989) and our results confirm this general trend. High density of colonising males and within-harem crowding lead gallery length to reduce noticeably, but the newly observed strategy of density-dependent regulation of egg population (fewer, well-spaced eggs in short galleries) can not help reducing space and resources for the brood. Spreading the reproductive effort over two generations may allow the beetle to reduce the risk of intraspecific competition, as there is a higher probability to find susceptible trees that can be killed with a lower attack density, releasing space to the colonizers. The contrasting values of attack density observed across generations and years may reveal the plasticity of the system, tending to optimize attack densities in relation to tree susceptibility and to limit mortality associated with overcrowding of broods.

Insects adapt their thermal responses to the habitat (Taylor 1981) adjusting phenology to maintain favourable match between themselves and the environment. For this reason it is expected that a significant impact of global warming may affect bark beetles performance both directly and indirectly, through the effects on community associates (symbiotic microorganisms and natural enemies) and host-tree vigour (Bale et al. 2002; Bentz et al. 2010). As the studied population becomes active at rather low temperature and has developed an appropriate matching with availability of susceptible hosts, this may lead to an increase in frequency and severity of outbreaks. Furthermore, the early emergence combined with the overwintering of many beetles outside of the breeding material may reduce the exposure to natural enemies associated with the bark, enhancing survival and allowing the population to achieve the high density required to successfully colonise trees.

Acknowledgements

We thank the foresters of the Regional Forest Service of the Belluno district for their precious assistance in field work. We also sincerely thank Tommaso Anfodillo, Fausto Fontanella and Roberto Menardi of the Centro Studi per l’Ambiente Alpino ‘Lucio Susmel’ of the University of Padova in San Vito di Cadore for providing meteorological data and simplifying field data collection. We also greatly appreciate the comments and suggestions made by Lorenzo Marini and Edoardo Petrucco Toffolo. The research was supported by the PRIN project, 200774ENMR: “Climatic change and Italian pine pests: a model study”.

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Table 1 Thermal sums calculated in each year from January 1st to the date of the spring flight peak considering thermal thresholds ranging between 0 and 10 °C

Spring flight peak	**Year**	**Thermal sum of daily mean temperature above 0-10 °C**										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
April 22nd	2007	423	338	268	211	164	129	100	74	54	39	26
May 20th	2008	483	389	309	241	184	136	96	64	37	19	7
May 13th	2009	400	333	273	220	173	133	99	71	48	34	20
	Mean	435.32	353.25	283.36	224.10	173.53	132.70	98.51	69.61	46.15	30.46	17.53
	± sd	42.96	31.17	22.76	15.01	9.71	3.24	2.19	5.20	8.61	10.33	9.32

Table 2 Mean density (± SD) per dm^2 of parent beetles and preimaginal stages observed in the four sampled generations (N = 21). Generation, year, and their interactions were tested by a two-way ANOVA for each variable

	Density/dm^2				**Two-way ANOVA**		
	2006/2007 generations		**2007/2008 generations**		***p-value***		
	F_2 2006	**F_1 2007**	**F_2 2007**	**F_1 2008**	**Generation**	**Year**	**Interaction Gen*Year**
Nuptial chambers (males)	6.7 ± 1.4	8.5 ± 0.8	7.3 ± 0.9	6.5 ± 1.0	ns	ns	< 0.05
Egg galleries (females)	17.0 ± 2.0	20.2 ± 1.5	17.8 ± 1.7	17.9 ± 4.0	ns	ns	Ns
Colonisation density (males plus females)	23.7 ± 3.4	28.7 ± 1.5	25.1 ± 2.6	24.4 ± 5.0	ns	ns	Ns
Harem size (females per male)	2.6 ± 0.3	2.5 ± 0.4	2.5 ± 0.2	2.8 ± 0.2	ns	ns	Ns
Eggs	170.9 ± 36.1	136.0 ± 26.6	145.7 ± 15.8	102.3 ± 33.5	< 0.005	< 0.05	Ns
Young larvae	138.3 ± 29.7	115.6 ± 23.6	123.6 ± 17.1	87.6 ± 32.0	< 0.05	ns	Ns
Mature larvae	121.0 ± 28.1	99.0 ± 14.9	110.3 ± 13.6	78.3 ± 31.3	< 0.01	ns	Ns
Pupae	95.3 ± 27.6	63.0 ± 15.7	82.5 ± 21.6	55.8 ± 18.5	< 0.01	ns	Ns
Pre-emerging adults	45.7 ± 17.9	16.0 ± 6.4	34.9 ± 11.5	15.8 ± 6.3	< 0.01	ns	Ns

ns = not significant

Fig. 1 Mean number of *I. acuminatus* caught per trap (± SEM) and daily maximum air temperature (dashed line) in 2007 (a) and 2008 (b). Horizontal line corresponds to 14°C. On the X axis are reported the dates of trap emptying and the period of trap checking before the beginning of flights

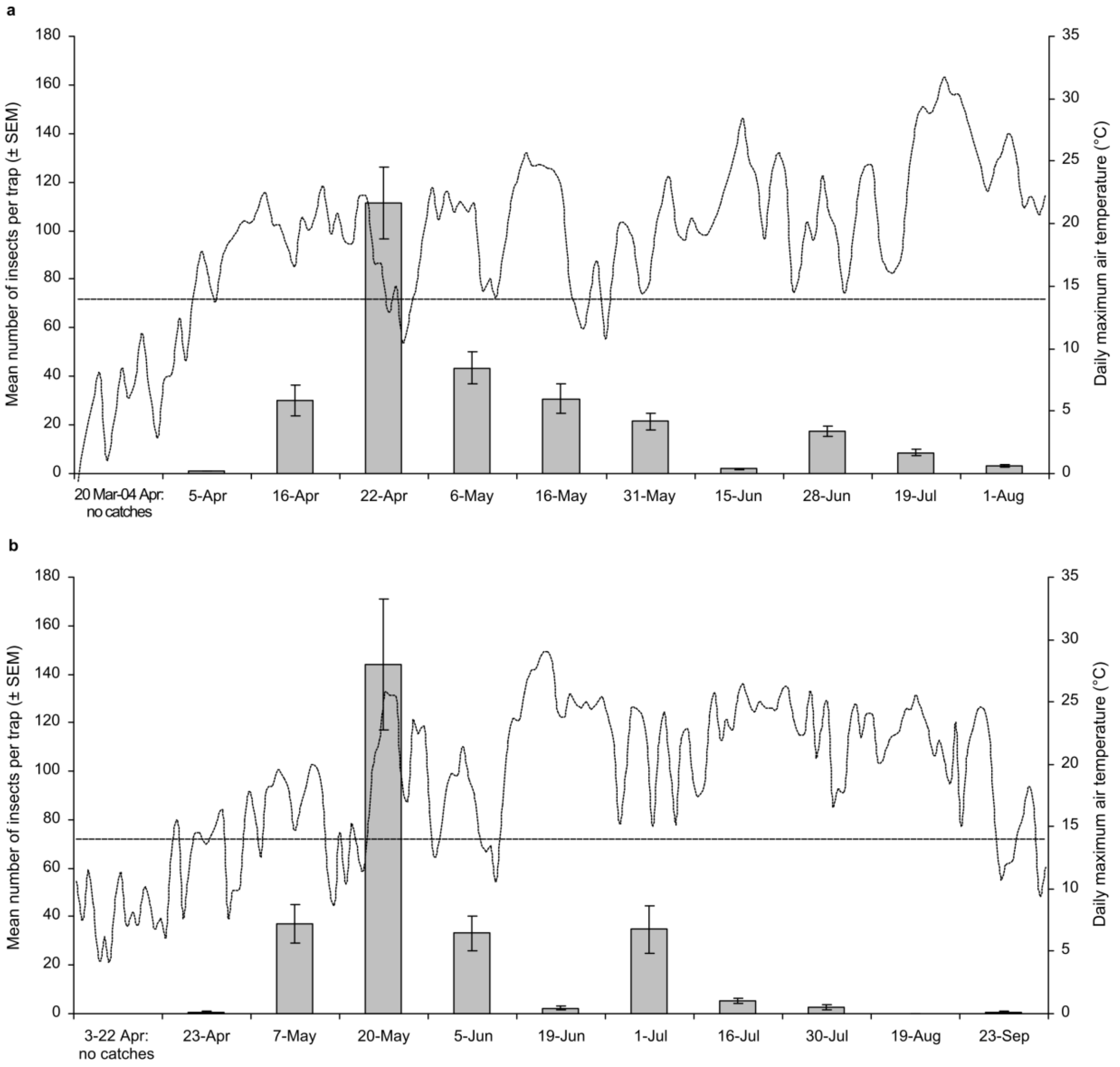


Fig. 2 Relationships between length of egg-galleries and density of colonising males (mating systems per dm^2) (N = 63) (a), number of eggs and gallery length (N = 21) (b), distance between eggs and eggs per egg gallery (N = 12) (c)

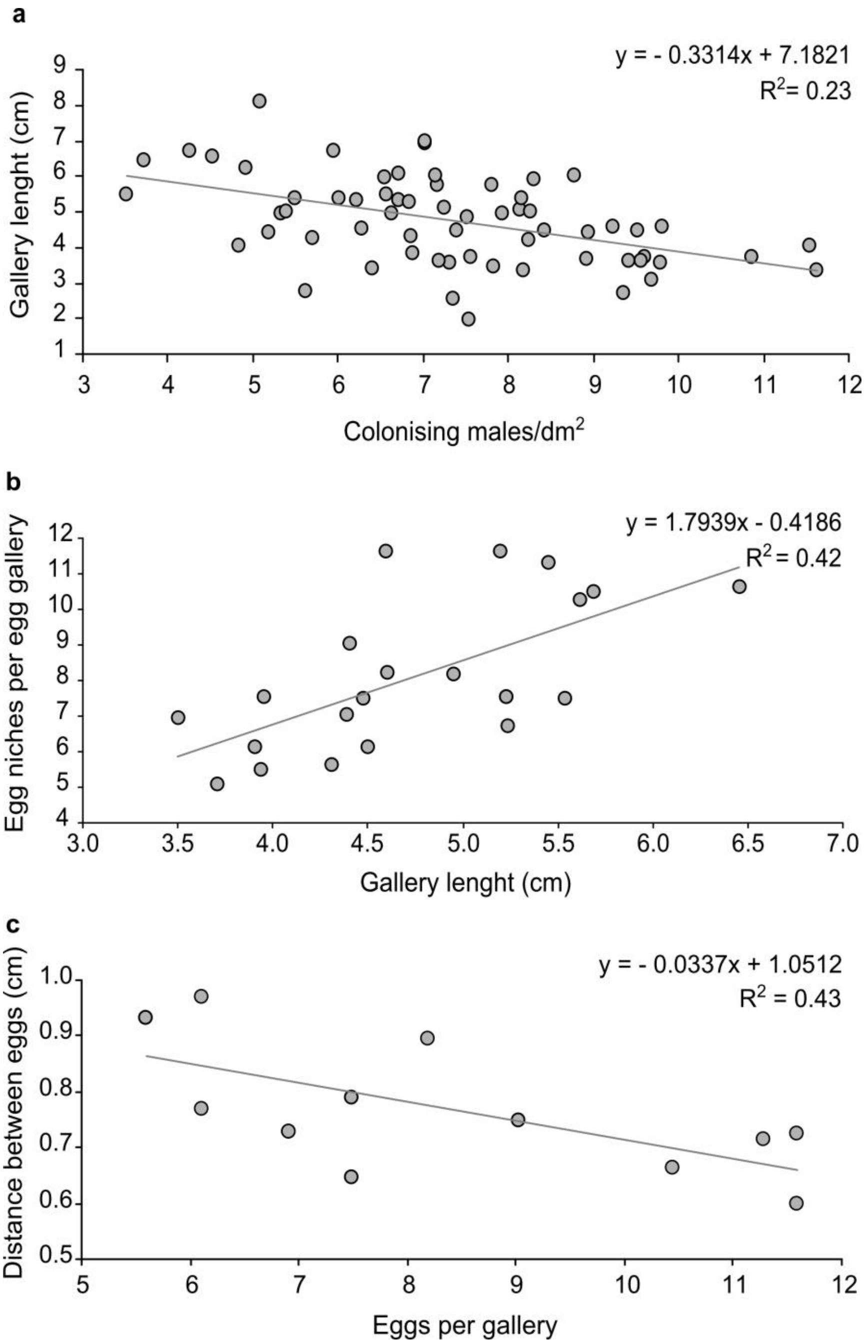


Fig. 3 Offspring production per female at different colonisation density (males and females per dm^2 of bark) (N = 21)

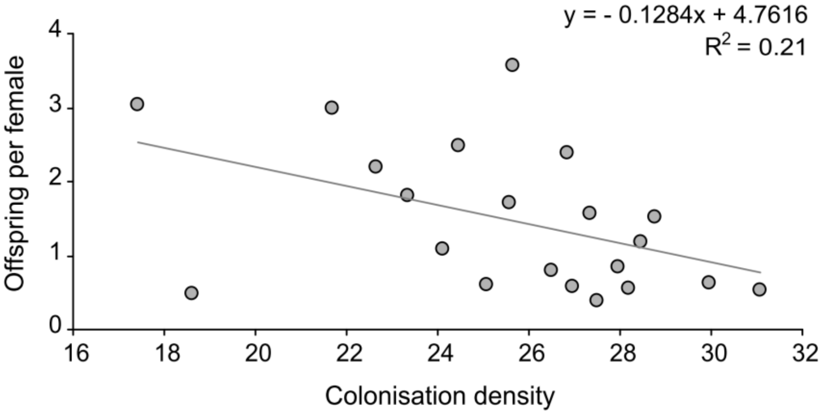
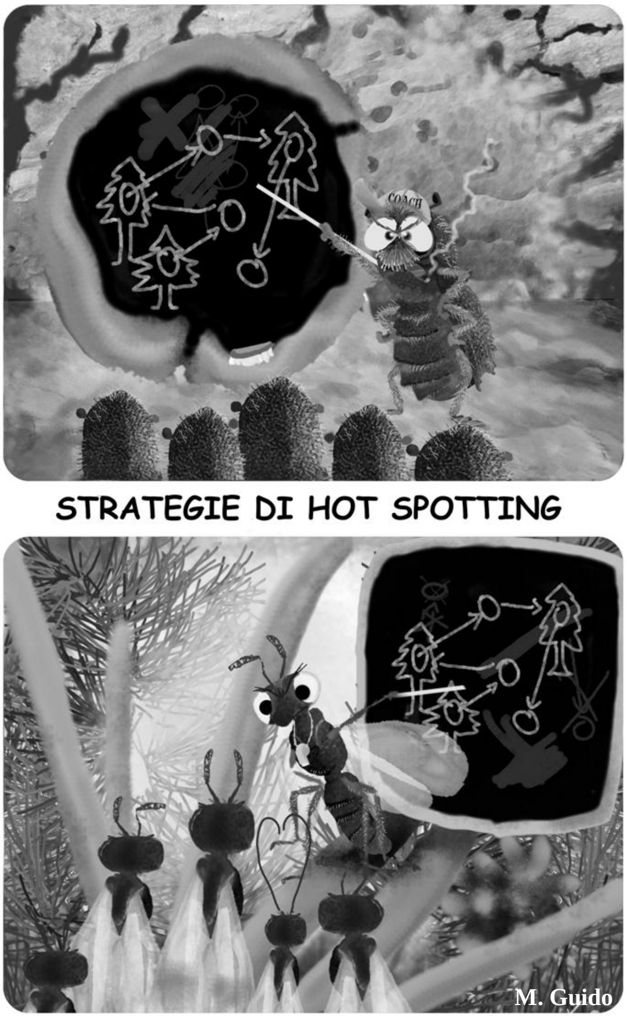


Fig. 4 Mean density (± SD) of individuals of each instar per dm^2 (N = 21)

Chapter II

Spatial-temporal dynamics of Ips acuminatus *infestations in the south-eastern Alps*



Ready for submission as:

Colombari F., Schroeder L.M., Battisti A., Faccoli M.

Spatial-temporal dynamics of Ips acuminatus *infestations in the south-eastern Alps*

I collected most of the data, analysed them and drafted the manuscript.

Abstract

- The population dynamics of the bark beetle *Ips acuminatus* in an outbreak occurred in *Pinus sylvestris* stands in the South-Eastern Alps was studied.

- Multiple-tree infestations (spots) were randomly distributed throughout the stands, varied in number and size with the phase of the outbreak and tended to have an annual life cycle.

- As the beetle was partially bivoltine at this latitude, the attack behaviour of the insect was seasonally dependent with beetles flying in spring and summer. In summer there was a short range dispersal leading to spot growth when emerging beetles attacked the neighboring trees. The number of newly attacked trees was proportional to spot size, but small spots (<10 trees) did not grow at all. In the next spring there was a long range dispersal leading to spot proliferation hundreds of meters away from the old spots. The 81.3 % of the new spots appeared within a 500 m radius of an old spot and, considering only the bigger spot classes (26-50 trees and >50 trees), source spot size and distance were significantly related.

- Thus, size of the spots influenced their evolution and dynamics in the year and between years and, in particular, small spots did not seem contribute to the spread of the infestations as the large spots did. The susceptibility of small spots to extinction, probably influenced by many interacting factors (e.g. bark beetle migration, depletion of resources, incidence of natural enemies) might be of value in management making decisions.

- Understanding landscape-scale spatial and spatial-temporal processes of bark beetle infestations is important when modelling bark beetle behavior and predicting where infestations will occur, although the coexistence of many site and stand variables must be considered.

Keywords: engraver beetle, spot dynamics, spot growth, spot proliferation, spot size.

Introduction

Bark beetle eruptions are known to be driven by a ‘complex suite’ of thresholds, feedbacks, and external forces characterizing the interactions across different levels of biological hierarchy and spatiotemporal scales: host tree, stand-mesoscale and landscape scale (Raffa et al., 2008).

The main variables that limit the distribution and abundance of bark beetles are climate and the availability of susceptible host type (Coulson, 1979). Other factors, such as concentrated beetle density, optimal symbiotic associations, and escape from natural enemies, may trigger outbreaks, as beetles are able to exceed the key thresholds at the different levels (Raffa et al., 2008). Because of the contemporaneous operation of many interacting site and stand variables, identification of specific combinations that consistently contribute to the incidence of infestation, and which might be of value in predicting where infestations will occur, has been difficult for most species. Furthermore, the conditions that contribute to stand susceptibility and suitability for bark beetle development obviously are not uniformly distributed either spatially or temporally, changing throughout the life of the stands (Coulson, 1979). However, understanding landscape-scale spatial and spatial-temporal processes of bark beetle infestations is important when modelling and/or predicting bark beetle activity and behaviour (Nelson et al., 2003).

Many beetle species have a very pronounced spatial patterning linked to the cooperative attack strategy they adopted, resulting in a ‘spot infestation’ (Turchin et al., 1998; Lieutier, 2004). When density becomes too high in the tree, beetles produce a repelling pheromone inducing the following (late) colonising beetles to attack nearby trees. In the new hosts the same processes occur, leading to a multiple-tree infestation (spot) (Turchin et al., 1998). Swarming beetles of the new generation disperse and initiate an infestation at a new location (spot proliferation) or enlarge the old infestation attacking other trees at the same location (spot growth) (Turchin et al., 1998; Safranyik, 2004; Robertson et al., 2007). The pattern of spot distribution is highly dynamic and tends to expand, as susceptible host trees are progressively depleted in the forest stand. The clumped distribution of the infested trees despite homogeneous stand conditions, however, leads to hypothesise that biotic interactions are involved in regulating the system (Turchin et al., 1998).

The current *Ips acuminatus* (Gyllenhal) (Coleoptera: Curculionidae, Scolytinae) infestations have been active in a valley of the south-eastern Italian Alps since 2005 (Colombari et al., 2008). The beetle is a wide-ranging insect, as it covers large part of the distribution of Scots pine (*Pinus sylvestris*), its main host (Novak, 1976). Despite this, damage due to this species was usually very low (Bakke, 1968). Only recently, as the increasing infestations and tree mortality (Forster and Zuber, 2001; Lozzia and Rigamonti, 2002; Wermelinger et al., 2008), *I. acuminatus* has been recognized as a pest in the European pine stands (Grégoire and Evans 2004), leading the attention on some unclear aspects such as biology and voltinism (Colombari et al., submitted).

With the present research work we aimed to improve the poor knowledge on *I. acuminatus* population dynamics. In particular, as host damage and reproduction by the insect occur primarily in well-defined patches (spots) (Forster and Zuber, 2001; Lieutier et al., 1988; Colombari et al., submitted), we wished to clarify the pattern of spot growth and spot proliferation over a five years period, corresponding to different phases of an outbreak. Spot dynamics was also related to voltinism, dispersal and spatial pattern of colonization.

Materials and methods

Study Area

Field data used in this analysis had been collected in a 25 km^2 Scots pine (*Pinus sylvestris*) forest located in the Dolomites (Italian Alps) (46°27’ N, 12°12’ E, about 1000 m a.s.l.). The terrain is mountainous, marked by steeples and pinnacles, with vertical walls and sheer cliffs separated by narrow and deep valleys. Scots pine (*Pinus sylvestris*) is the dominant tree species, which covers almost the 100% of the forested land, followed by Norway spruce (*Picea abies*) and dwarf mountain pine (*Pinus mugo* subsp. *mugo*). Mature Scots pine stands (>100 years) are located on south-western facing slopes and have a mean density of about 300 stems per hectare.

Monitoring of infestation spots

As *I. acuminatus* was found to be bivoltine in the study area., host trees are colonised in spring and summer by hibernating and new generation beetles, respectively (Colombari et al., submitted).

Infestation spots were monitored from autumn 2006 to autumn 2010 as soon as red-crowned trees became evident after colonisation. Monitoring procedure was divided into two stages. The first survey stage determined the indicative location, number and size of infestations within the area, by observing pine stands from the opposite slope of the valley. During the second stage, the ground surveys located each infestation to record the spatial location of the groups of attacked trees by positioning a GPS in the centre of the spots, and to assess average tree dbh., height, age and density within the spots. Red-crowned trees of each spot were examined for signs of *I. acuminatus* recent infestation and predominant beetle life stage occurrence, and counted. During the 2006 survey about 500 old infested trees, clumped in small multiple-tree spots, were observed.

The two-stage monitoring procedure described was completely followed in autumn 2006, in both summer and autumn 2007, and in summer of all the following years, whereas in autumn 2008, 2009 and 2010 only first-stage information were collected.

As infestation spots were generally circular and their development usually occurs by addition of newly attacked trees along one or more fronts, a 12 m maximum distance (United States Department of Agriculture, 2003) was chosen between one attacked tree to another for being recorded as belonging to the same group. Over this distance, trees were considered to belong to different spots.

Considering outside interferences that should have acted in spot dynamics during the surveyed period, it is to mention a sanitation felling conducted in autumn 2007 (last September - October). Most of the spots in the area were cut (about 90%) applying the ‘cut and remove’ method. In detail, the treatment was applied on 3,370 recently infested and 1,194 apparently unattacked trees, these latter belonging to the spots or to the buffer strips cut around the edge of the spots. In a recent study the effectiveness of the treatment has been analysed (Colombari et al. unpublished data), assessing that spot dynamics was not influenced by the cutting because the infested trees removed at the end of the summer had been already left by a great part of the beetles (96 %) that spend the winter elsewhere (Colombari et al., submitted).

Statistical analysis

Differences between years were tested by a one-way ANOVA for each variable (tree dbh, height, distance, number of trees per spot). To fit the assumptions of the ANOVA, variables were log transformed, if necessary. Significance was determined at $p<0.05$. Moran's I was calculated to test whether the spot size was spatially autocorrelated. Regression analysis was used to test relationships between pair of continuous variables. The analyses on distances between old spots (year t-1) and new ones (year t) were based on the assumption that trees infested at a new location were attacked by beetles swarmed from the nearest field spot (Jakuš et al., 2003; Robertson et al., 2007).

Statistical analyses were performed using the software programs R (version 2.7.0), Statistica 8 (Statsoft, Inc 1993) and the application SAM - Spatial Analysis in Macroecology. Mean values are reported ± standard error, unless otherwise specified.

Results

General trend of the spots in the five years

Totally, field data were collected for 199 spots. The pattern of colonization resulted mainly in clumping of dead trees scattered over the valley (Fig. 1), as very rarely isolated infested trees were observed (6 cases in five years). Distance between spots of the same year varied significantly between years ($F_{(4,4719)} = 29.63$; $p<0.00001$). A contraction of distances was observed in 2009, followed by a sudden new expansion in 2010 (Fig. 1). No changes in spot elevations were observed between years.

The number of spots in the whole area slightly decreased from 2006 to 2008 (-15.0 %) and, after a sharp fall to the lowest value registered in 2009 (-72.5 %, 14 spots), moderately increased in the following year (+ 33.3 %) (Fig. 2), whereas the number of recently infested trees peaked in 2007 and then fell down suddenly (Fig. 2). As a result, mean spot size reached the highest values in 2007 (65.6 ± 11.0; $F_{(4,\ 194)} = 12{,}32$, $p<0{,}00001$) and then decreased, remaining more or less constant (15.5 ± 1.8, 11.0 ± 2.0, 13.0 ± 3.3 in 2008, 2009 and 2010, respectively) (Fig. 2).

The size of the spots varied greatly, ranging from 2 to 390 killed trees with a maximum radius of about 70 m. No spatial autocorrelation was found in any of the five observed years ($p>0.5$). Following Ayres et al. (in press) spots were divided into four size classes (1-10 trees, 11-25 trees, 26-50 trees, >51 trees) to analyse spot evolution and dynamics in the year and between years.

The number of medium size spots (11-25 trees) was very similar between years, whereas the following class (26-50) gradually decreased (Fig. 3). Strong differences were observed in small (1-10 trees) and large (>51 trees) spots: small spots were abundant in 2006 and particularly in 2008, 2009 and 2010 but very scarse in 2007 when large spots, doubling from the previous year, were the predominant class (Fig. 3). After this peak, large spots decreased strongly and were completely absent in 2009. A slight increase (+ 4.8 %) was registered in 2010.

During the infestation diameter and height of attacked trees decreased significantly between years ($F_{(4,194)} = 4.57$, $p< 0.005$; $F_{(4,194)} = 8.72$, $p< 0.00001$, respectively), reaching the lowest values in 2009 (17.7 ± 1.4 cm and 7.2 ± 0.3 m) and then increasing again, although not significantly, in 2010 (19.9 ± 1.3 cm and 10.1 ± 0.6 m).

Pattern of infestation

As a result of the attacks of the first generation during the summer, spot enlarged their size (spot growth) whereas new spots occurrence (spot proliferation) was observed only in spring, after the swarming of the overwintering adults (Fig. 4).

In summer 2007 the 62.3 % of the spots enlarged including the neighboring trees and increasing 40% the number of infested trees in the existing spots. Moreover, the enlargement was directly proportional to the size of the source spot ($R^2 = 0.30$, $F_{(1,31)} = 13,26$, $p<0.001$) (Fig. 5) but spots of the 1-10 trees class did not grow at all. This pattern of continuous spot growth was not so evident in summer 2008, when number of infested trees increased only by the 10%, and it was totally absent in 2009 and 2010.

The distance between an old spot (year t -1) and the nearest new one (year t), ranging from 11.4 to 3768.0 m (494.0 ± 70.3), varied significantly between years ($F_{(3,135)}= 26.02$, $p<0.00001$) and reached the highest value in 2010. The 81.29 % of the new spots appeared within a 500 m radius of an old spot. After a gradual increase occurred in the previous years, spots belonging to the higher distance classes (300-400 m, 400-500 m and >500 m) disappeared in 2009, whereas the number of spots of the lower classes (0-100 m, 100-200 m) greatly increased (fig. 6). In 2010 the trend changed suddenly, with a peak in the distance class >500 m, whereas the lower and medium classes reached the minimum values.

Analysing all spot sizes, no relationships were found between the size of a new spot and the size of the nearest old spot whereas, considering only the bigger spot classes (26-50 trees and >50 trees), size of the source spot and distance were significantly related (2006/2007 $R^2 = 0.16$, $F_{(1,25)} = 4,63$, $p<0.05$; 2007/2008 $R^2 = 0.17$, $F_{(1,23)} = 4,68$, $p<0.05$) (Fig. 7): increasing the number of trees in the source spot, the distance between this spot and the nearest new one increased, but also in this case the size of the new spot was not related to the distance. As spots bigger than 25 trees were very few in 2008 and 2009, the relationships were analysed between source spots and new spots in 2006-2007 and 2007-2008. We tested the interaction between year and size of source spot on distance, obtaining that the trends were similar in the two years ($p>0.05$).

Discussion

Our results show that *Ips acuminatus* dispersal and attack behaviour change with the generation, leading to spot growth within the year and to spot proliferation between years. The pattern also varies with the different stages of the outbreak and with the size of the spots, as small spots do not seem to contribute to the spread of infestations as the large spots do.

Previous researches (Safranyik et al., 1989; Safranyik et al., 1992) described the possible occurrence of three different spatial patterns of infestation resulting from beetle dispersal. Short range dispersal flights lead to spot growth, occurring when beetles swarm from the infested trees of a spot and attack the nearby trees located along the edge of the spot. On the contrary, long range dispersal flights lead to spot proliferation, occurring when beetles initiate an infestation at a new location. Lastly, there could be a third kind of dispersal consisting in movement of beetles between spots, not considered in our analyses.

We observed that spot growth and spot proliferation are two distinct processes involved in the expansion of areas attacked by *I. acuminatus*, occurring separately during the dispersion of the first and second generation respectively. Spot growth occurs only in summer, when first generation bivoltine adults swarm and colonise the neighboring uninfested trees, expanding the size of the spot, whereas spot proliferation occurs only in spring, when overwintering adults disperse and aggregate to form new infestations hundreds of meters away from the old spots. Beetles swarming in spring represent adults of both first and second generations, as about 50% of *I.acuminatus* first generation adults are monovoltine (Colombari et al., submitted). Thus, before spots become inactive, two beetle generations can emerge. The spatial distribution linked to spot proliferation is already known in the *Ips* genus (spot spreading described for *I. typographus* in Wichmann and Ravn, 2001 and in Jakuš et al., 2003) and the coexistence of both spot growth and spot proliferation in each generation has been assessed in *Dendroctonus ponderosae* Hopk. (Robertson et al., 2007), whereas a seasonally dependent attack behaviour is typical in the southern pine beetle (SPB, *D. frontalis* Zimmermann). During the summer spots may continue to grow in size under the consecutive attacks of several SPB generations, whereas in the following spring spots cease to be active as beetles emerge and disperse to form new aggregations (Coulson, 1980; Ayres et al., in press), randomly distributed with respect to the old ones (Billings and Pase, 1979).

Long and short range dispersal flights have different importance on the spatial dynamics of an infestation: the former, spreading the population throughout the area, lead beetles to explore new distant stands (Billings and Pase, 1979) where they could find better resources (‘higher quality trees’, Safranyk and Carroll, 2006), whereas the latter, maintaining the population on the nearby trees (Hedden and Billings, 1979), may allow greater investment of energy in reproduction (Elkin and Reid, 2005) reducing the uncertainty of dispersal (Gara, 1967). Furthermore, we found that the spatial distribution of patches of attacked trees is influenced by the phase of the outbreak. We can recognize in 2005 the beginning of the epidemic phase, as clusters of infested trees became evident during the surveys, and in 2007 the peak of the outbreak, as the occurrence of successful mass attacks against large diameter trees and the great number of large size spots (> 51 trees). On the contrary, the number of such spots was very low in the declining phase when most of the spot belonged to the 1-10 trees class. In our study area spot growth occurred strongly in 2007, moderately in 2008 but it was absent in 2009 and 2010. As the enlargement is directly proportional to the size of the source spot and spot of the 1-10 trees class do not grow at all, the decrease in the spot growth phenomenon could be related to the observed reduction of beetle population and, consequently, of spot size during the declining phase (Ayres et al., in press). However, the failure of the colonization does not necessary mean the total absence of a second generation, as parent beetles might colonize pieces of branches or isolated standing or felled weakened trees.

Spot size influences also spot proliferation, as we found larger spots are able to spread individuals at longer distance. This might be explained, following the theory of ‘free-flight’ behaviour, as a tool to avoid overcrowding (Hughes et al., 2006) because some bark beetle species require this kind of period before they become responsive to pheromones (Borden et al., 1986). Studies on *I. typographus* suggest that dispersal patterns may be altered by free flight periods (Helland 1984; Helland 1989). In *D. ponderosae* ‘an indirect evidence of the free-flight effect on dispersal is given by the fact that some beetles fly past nearby attractive sources (Safranyik et al., 1989), and that infestations spread before all the hosts within an infested area are depleted (Mitchell and Preisler, 1991; Borden, 1993)’ (Hughes et al., 2006). Moreover, during the declining phase we observed a contraction of distances between old and new spots: with the reduction of the beetle population, probably the necessity of flying farther in order to avoid overcrowding ceases and the nearest susceptible hosts are attacked. This hypothesis could be also confirmed by the fact that spot proliferation usually occurs mainly within a radius of 500 m, suggesting the presence of a traversable landscape in which beetles are allowed to move between patches of susceptible hosts (Barclay et al., 2009).

It is not improbable indeed to have from 12 to 25 susceptible host trees per km^2, considering all the conditions that may determine the occurrence of stressed or disturbed trees, such as diseases, fire, lightning (Coulson, 1979) or, as in our study site, rockfalls. These ‘reservoirs’ may maintain small populations in non-outbreak years and may explain the rapid appearance and evolution of bark beetle infestations as soon as suitable climatic conditions occur, thus excluding long distance migration as necessary factor involved in outbreak explanation (Coulson, 1979).

Size of the spots influences the spatial pattern of colonization and its evolution in the year and between years. In particular, small spots do not seem to contribute to the growth and spread of the infestation, confirming findings of previous researches. Extinction of small spots might be explained hypothesizing a strong impact of natural enemies (Reeve, 1997) or considering the low possibilities that small populations of attacking beetles have in overwhelming tree defenses (Berryman et al., 1985) (Ayres et al., in press). However, small spots can contribute to maintain during the endemic phase a low level of beetle population ready to start a new spread as soon as conditions are favourable, as demonstrated by the increase in the number of large spot and distances in 2010, after rockfalls recorded during winter.

The susceptibility of small spots to extinction, is probably under the influence of many interacting factors (e.g. bark beetle migration, depletion of resources, incidence of natural enemies) and might be of value in predicting population trend. As *I. acuminatus* resource exploitation is based on reaching defined high attack thresholds similar between years and generations (Guérard et al., 2000; Colombari et al., submitted), number and size of the infestation spots may depend on the interactions between the availability of susceptible hosts and the activity of natural enemies.

Acknowledgments

We are grateful to Valerio Finozzi and Edi D’Ambros (Regional Forest Services) for helping the surveys and the trapping and providing precise data on the cutting. A special thanks to Attilio and Gino for the precious practical help in all field activities. We also greatly appreciate the logistic support in sampling activities given by Tommaso Anfodillo, Fausto Fontanella, Roberto Menardi of the Centro Studi per l’Ambiente Alpino ‘Lucio Susmel’ of the University of Padova in San Vito di Cadore. Lastly, we thank Edoardo Petrucco Toffolo and Lorenzo Marini for the constructive comments on the manuscript.

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Figures

Fig. 1 Maps of the spot distribution in the five observed years: reciprocal distance between spots of the same year varied significantly between years ($F_{(4,4719)} = 29.63$; $p<0.00001$). The coordinate system used is UTM.

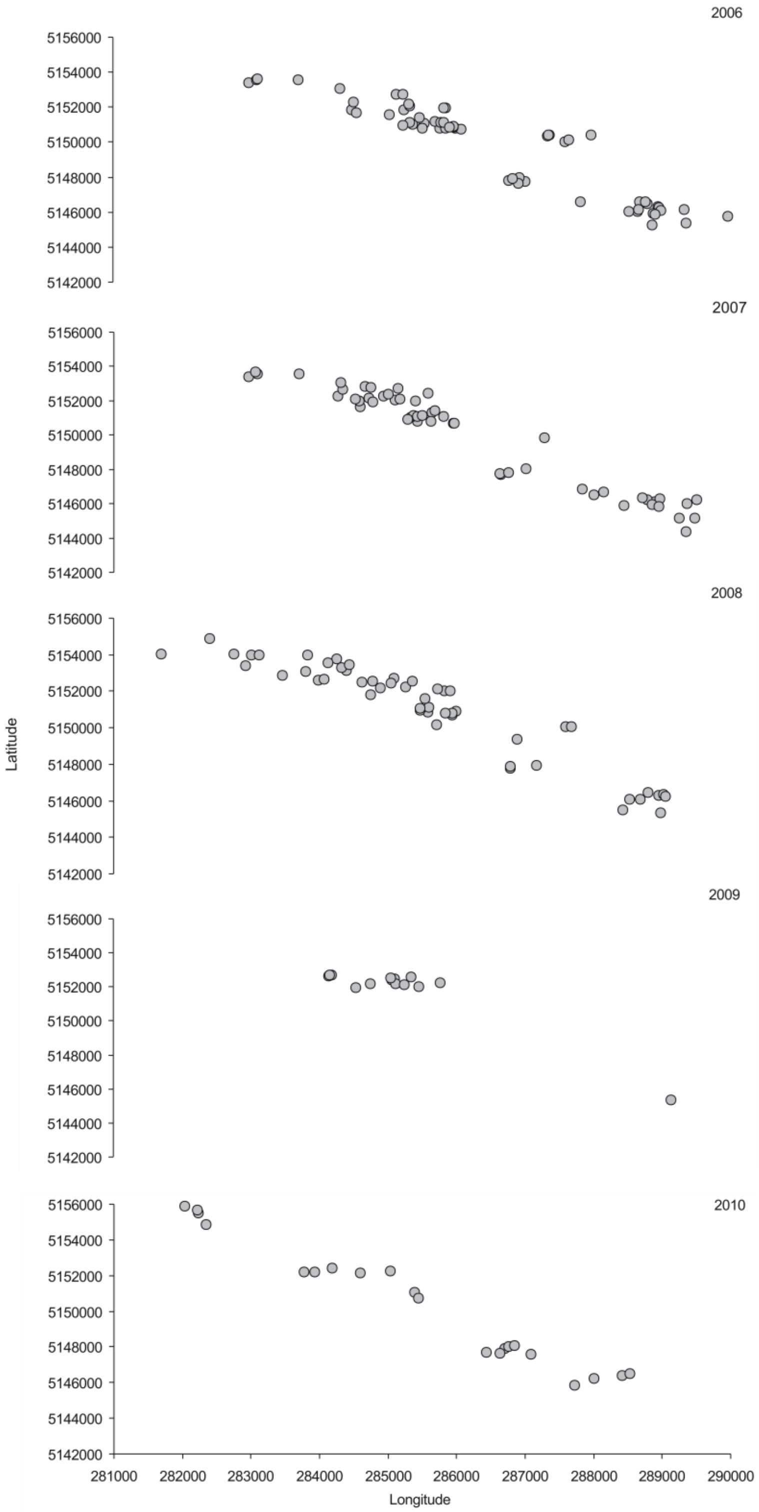


Fig. 2 Number of infested trees, spots and trees/spot (± SEM) in the five observed years.

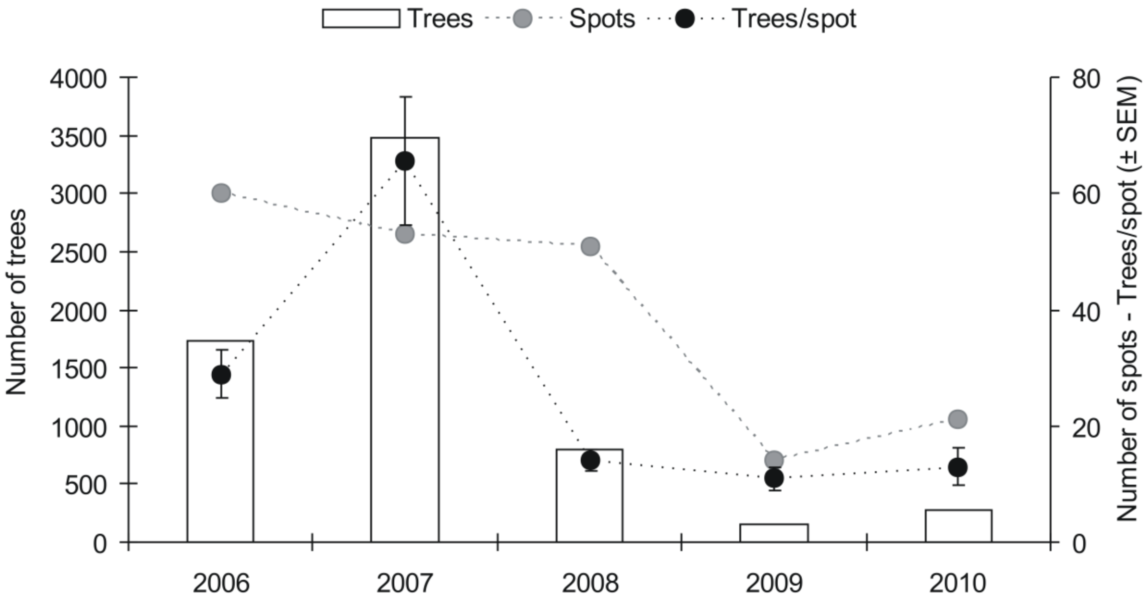


Fig. 3 Percentage of spots in each size class during the five observed years.

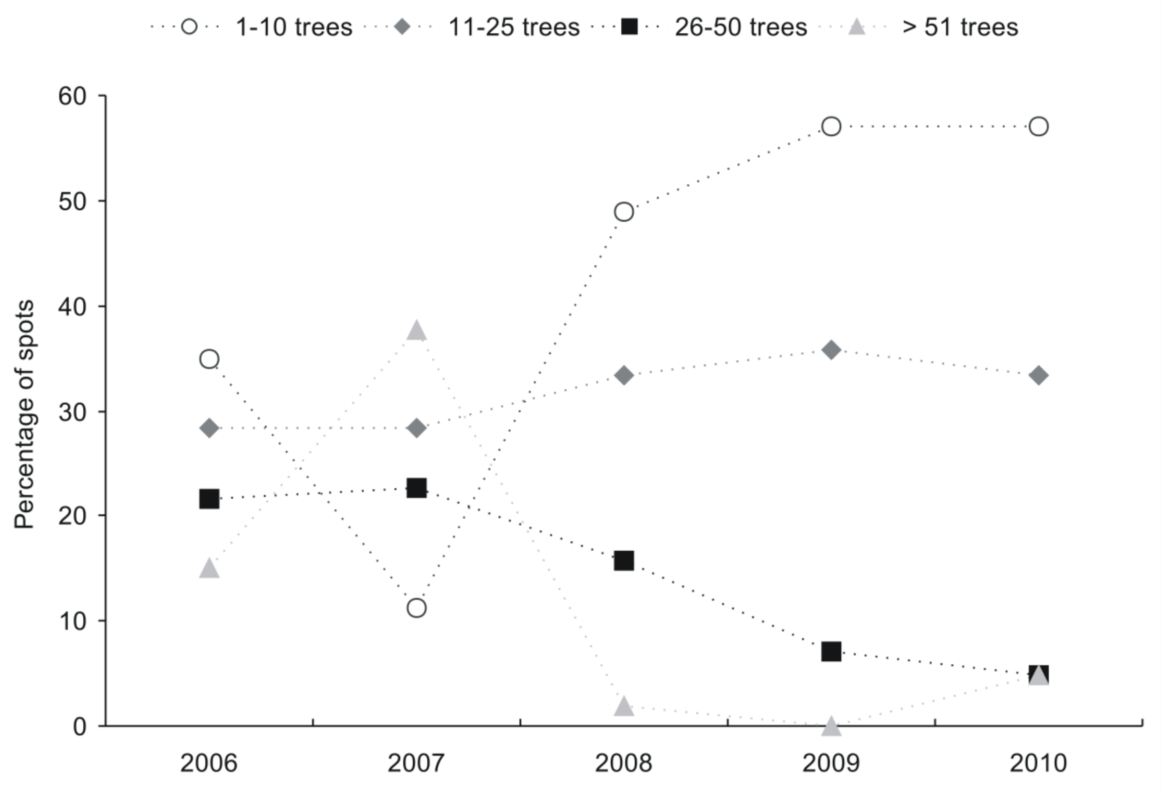


Fig. 4 Spot proliferation (**a**), occurring in spring after the swarming of the overwintering adults, and spot growth (**b**) occurring in summer after the swarming of the F1 bivoltine adults.

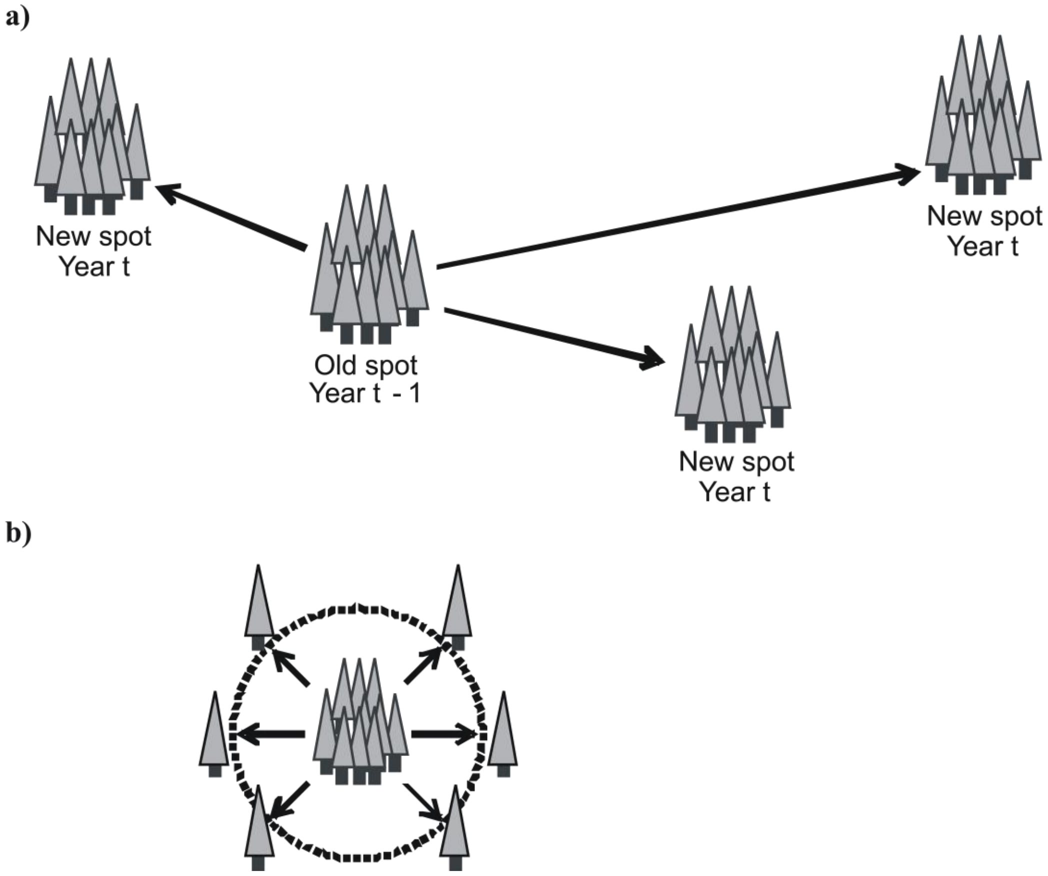


Fig. 5 Relationship between size of the source spot (number of trees infested in spring) and spot growth (number of neighbouring trees infested in summer) in 2007 ($R^2 = 0.30$, $F_{(1,31)} = 13,26$, $p<0.001$).

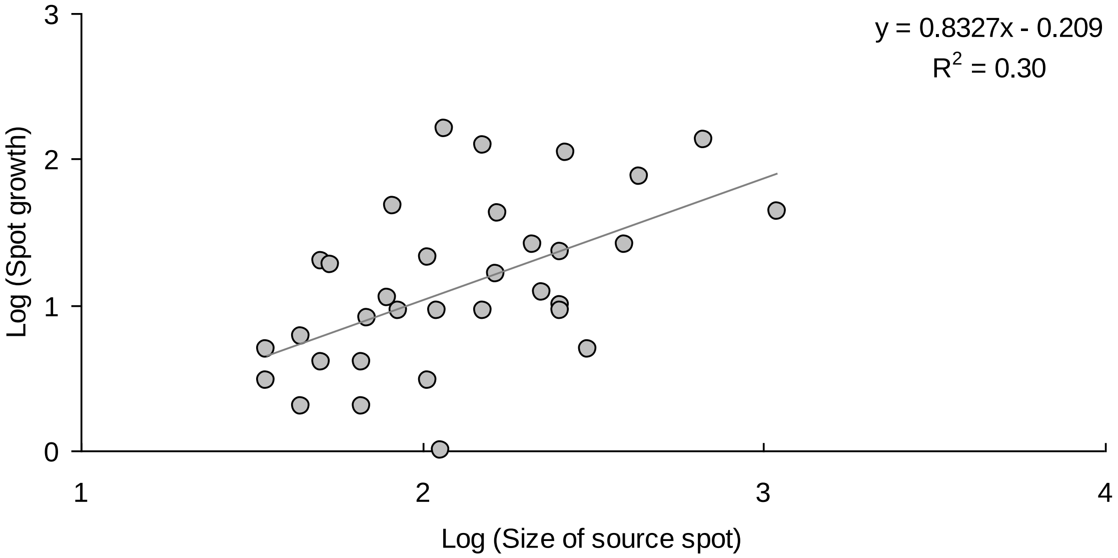


Fig. 6 Percentage of spots in each distance class (m) during four consecutive years.

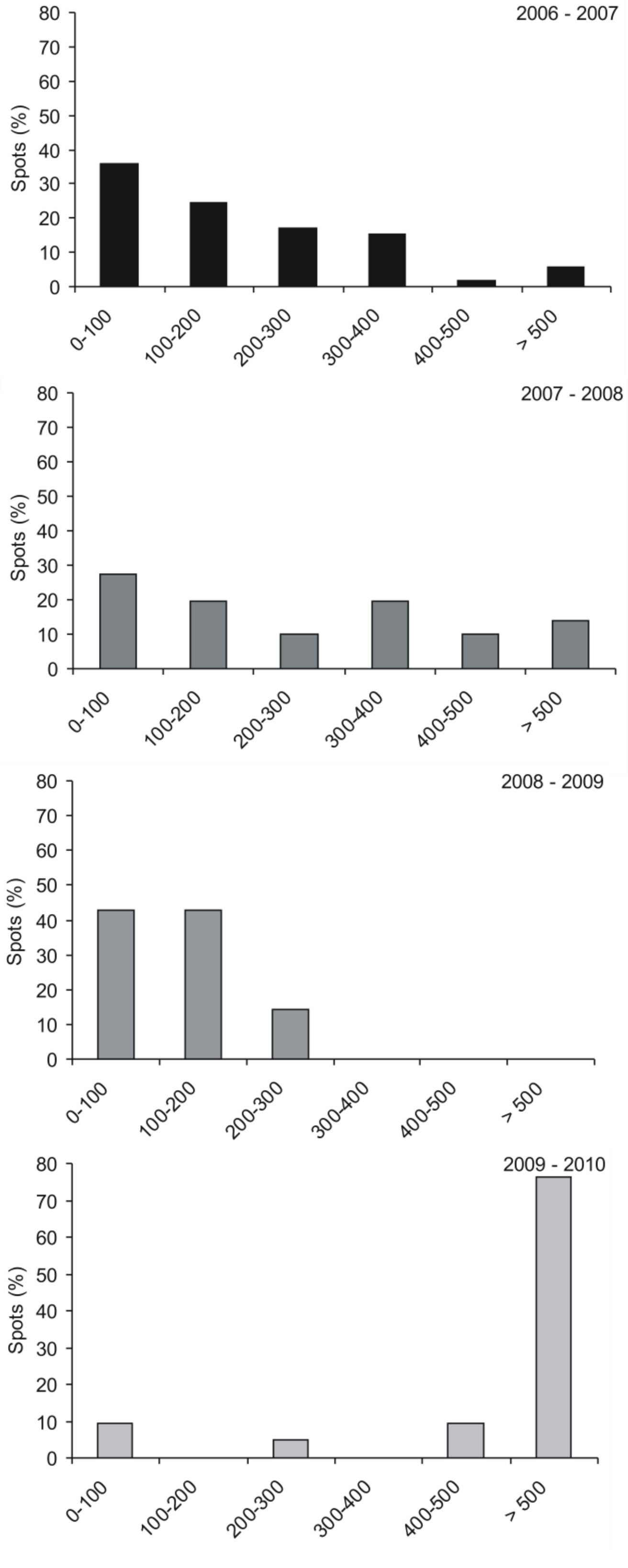
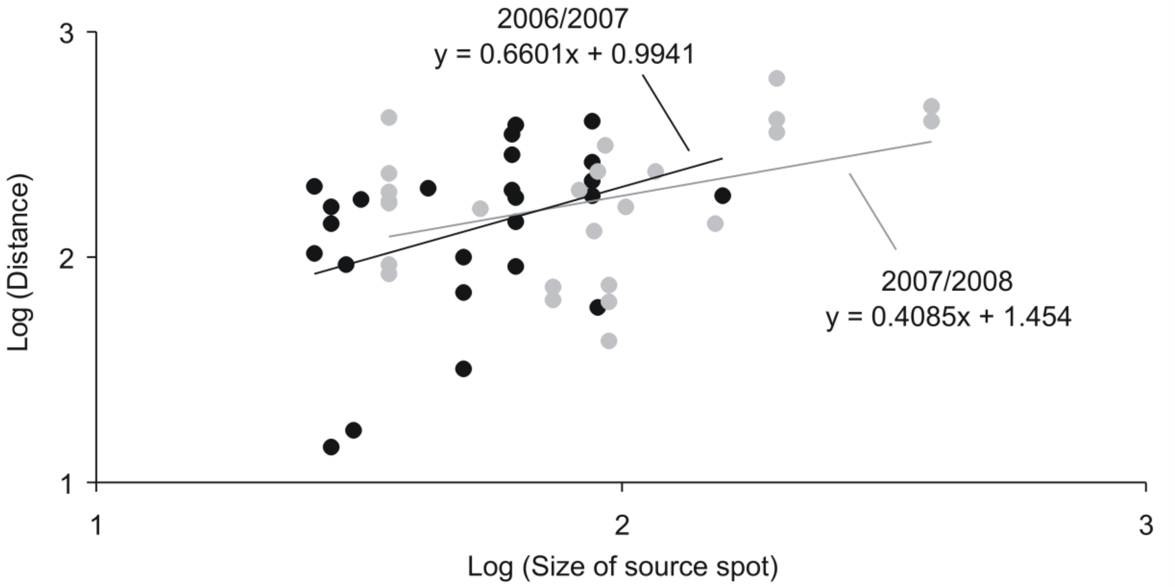


Fig. 7 Relationship between size of the source spot (number of trees) and distance (m) from source spot to new spot (2006/2007 $R^2 = 0.16$, $F_{(1,25)} = 4,63$, $p<0.05$; 2007/2008 $R^2 = 0.17$, $F_{(1,23)} = 4,68$, $p<0.05$).



Chapter III

Effects of a sanitation felling *on* Ips acuminatus *population and infestation dynamics*

Ready for submission as:

Colombari F., Schroeder L.M., Battisti A., Faccoli M.
Effects of a sanitation felling on Ips acuminatus *population and infestation dynamics*

I collected most of the data, analysed them and drafted the manuscript.

Abstract

- During an *Ips acuminatus* (Gyllenhal) outbreak that has spread in the Dolomites (Valle del Boite, Belluno district) since 2006, we evaluated for the first time the effects of a short-term suppression strategy on spot dynamics and damage level.
- The methods of ‘cut and remove’ was applied during the epidemic phase of the outbreak in order to destroy all the infested trees and the overwintering beetles inside them. One smaller part of the outbreak area was excluded from the suppression cutting and used as a control.
- The main apparent effect of the treatment was a reduction of the mean size of the spots in the treated areas during the first year after the treatment whereas no major differences were observed two years after the cutting.
- Nevertheless, other interacting factors seem to be involved in spot dynamics and evolution as demonstrated by the absence of new infested spots in the control area two years after the treatment. In particular, size of the spots has an important role in spot extinction.
- Considering that part of the beetle population does not overwinter under the bark and the absence of any kind of symptom other than change in colour of the crowns, evident late in the season, control of *I. acuminatus* outbreaks seems to be very difficult as sanitation cuttings can not be applied in the proper period.

Key words: engraver beetle, damage level, sanitation felling, spot dynamics.

Introduction

As scolytid bark beetles (Coleoptera: Curculionidae, Scolytinae) are the most damaging insects attacking conifers worldwide, the great economical importance of these forest insects led to develop a variety of mechanical, cultural, biological, and chemical techniques to control the problem. The degree of employment of each strategy varied with the type of pest and, particularly with the damage rating of the pest (Grégoire and Evans, 2004). The main tools used in reducing bark beetle-caused tree mortality includes sanitation felling of newly infested trees, and mass trapping either by trap trees or by pheromone traps (Niemeyer, 1997), as the high dependence of some bark beetles species on aggregation pheromones attractive to both sexes may allow an intensive trapping (Koul et al., 2004) that prevents adults from reaching and mass attacking susceptible host trees (Grégoire and Evans, 2004).

Nonetheless, sanitation/suppression treatment is the traditional and most used reactive measure to remove infested and green susceptible trees from an area of active beetle infestation (Hansen et al., 2010). Whereas long-term cultural practices (e.g. thinning) attempt to reduce future mortality by improving stand conditions and increasing, in this way, the ability of living trees to defend themselves (Grégoire and Evans, 2004), short-term suppression strategies are applied as expedited treatments to limit the negative impacts of ongoing outbreaks in high-value areas (WFLC, 2009). Cutting and removing the infested trees is about the only feasible course of action to follow if direct control is needed to reduce an infestation of beetles in a forest situation. The effectiveness of this last kind of treatments mostly depends on beetle mortality and/or reduction of tree mortality, at the level of the individual tree and spot (Hedden, 1979). Nevertheless, it is not simple to evaluate the success of a treatment, as multiple naturally occurring factors may be involved in reducing an outbreak (Foltz, 1979).

An outbreak of the pine engraver beetle *Ips acuminatus* (Gyllenhal) (Coleoptera: Curculionidae, Scolytinae) has been active in the south-eastern Italian Alps since 2005, when about 500 attacked trees, clumped in small multiple-tree spots, were observed (Colombari et al., 2008). A survey, carried out during the autumn 2006 in order to assess the evolution of the outbreak, revealed a 3- fold increase in the number of infested trees. Consequently, an intensive monitoring of infestation spots and beetle population started, revealing a wide spread of the attacks in 2007. The beetle colonises mainly the upper part of the tree (bark thickness of 2-4 mm) and performs two generations per year, with trees attacked in April-May and late June-July (Colombari et al., submitted). The infested trees become evident about two months after the attacks, when crowns turn reddish, and then die in a short time. Because of the high landscape value of the stands and their important role in soil protection, a sanitation felling was applied in autumn 2007, with the only exception of a small part of the outbreak area, as means for limiting damage and preventing further expansion of the beetle population.

The increasing importance of the insect in the Scots pine decline recently observed in the Alps (Wermelinger et al., 2008) and the expected intensification in frequency and severity of outbreaks in a climate-change perspective (Colombari et al., submitted), lead to consider the possible tools useful in limiting tree mortality due to this species. With the present research work we aimed to evaluate the effectiveness of sanitation felling comparing spot dynamics and damage level in treated and untreated areas over a four years period. Moreover, we also tried to assess if trapping could be a useful tool to forecast the future degree of infestations.

Materials and methods

The Study Area

The study area consist of a 25 km^2 Scots pine (*Pinus sylvestris*) forest located in the Boite valley, in the south-eastern Italian Alps (Dolomites, Belluno district) (46°27’ N, 12°12’ E, about 1000 m a.s.l.). The terrain is mountainous and Scots pine is the dominant tree species, which covers almost the 100% of the forested land, followed by Norway spruce (*Picea abies*) and dwarf mountain pine (*Pinus mugo* subsp. *mugo*). Mature Scots pine stands (>100 years) are located on south-western facing slopes and have a mean density of about 300 trees per hectare.

Monitoring of infestation spots

During a four years period (from autumn 2006 to autumn 2009) recently formed infestation spots, detected as a result of discoloration in crown foliage of attacked trees, were inspected from the ground. As trees may be killed twice a year (first attack wave by hibernating beetles and second wave by new generation beetles from the current year), surveys were conducted twice too (in early summer and autumn) to match these colonization events (with the exception of 2006 when the survey was only conducted in the autumn).

In each spot red-crowned trees were examined for signs of bark beetle attack and an inventory of all trees, infested and healthy, was taken. Previously infested trees (killed one to two years earlier) were discerned from recently killed trees (killed in the same year) by having grey instead of yellow to reddish foliage, whereas old infested trees (killed more than two years earlier) did not have needles at all. For each category, trees were counted and the average dbh, tree height and age in the spot were measured.

Monitoring of population densities with traps

Monitoring of relative population densities was performed by Multi-funnel traps (Witasek®) baited with an species-specific pheromone lure (Acuwit®, Witasek) in 2007, 2008 and 2009. The traps were set out early in the spring (late March or April) in the centre of spots of killed trees from the previous year. In 2009, 29 active spots were monitored by 65 traps (up to 7 traps per spot), whereas after the sanitation felling, traps were set in both treated and control area in order to evaluate the possible effects of the treatment. In the treated area, 12 and 7 traps were placed in 2008 and 2009, respectively. In control area, 3 traps were placed in both 2008 and 2009. The pheromone dispensers were replaced once after about two months. Twice a month the traps were emptied, until the flights ceased (August or September depending on the year). All trapped *I. acuminatus* were counted and sexed. Number of beetles caught by traps was averaged for each location (treated and control areas) and year considering both the entire trapping period and the two flight periods separately (mean trap catches of the spring and summer swarming flights).

Sanitation cutting of killed trees

Cutting of killed trees was conducted in the autumn (end of September – early November) of 2007 with the aim of reducing the damages the following year by removal of hibernating beetles. A smaller part of the outbreak area, at the northern edge, was left untreated as a control (Fig. 1). Mean elevation of infestation spots was similar (1,290.1 ±

21.4 m in the control vs 1,255.8 ± 38.6 m in the treated) as well as temperature, soil and stand conditions.

Within the treated area all spots (48) were cut, with the only exception of two unapproachable spots located on the top of cliffs. In each spot, all the recently killed trees (3,370), and apparently unattacked trees (1,194) were cut whereas trees that had died for more than one year ago were left in most of the cases. In addition, a buffer strip of apparently uninfested trees around the spot was cut (cf. Billings, 1980). Strip width varied with the size of the spot, corresponding to about three-four concentric rows of trees in larger spots. As mountain slopes are very steep and, for the most part, without forest tracks, boles and branches of cut trees were separated, transported by helicopter to nearby open areas and there destroyed by chipping. The completion of all the phases took about one month, ending on early November.

Estimation of the size of colonizing and hibernating population of beetles in killed trees

Eighteen spots of trees infested by *I. acuminatus* were randomly selected in late June (six spots) and October (six spots) of 2007 and in June of 2008 (six spots, three in the treated area and three in the control), as soon as crowns faded after the attacks. In each spot three trees were felled and diameter at breast height (1.3 m above ground), total tree height, height and diameter at the beginning and at the end of the insect colonization points, were recorded. In addition, in 2007, on one tree per spot (plus one, N=13) the Total Colonised Bark Surface (TCBS) was calculated by measuring also sample branches of each whorl. TCBS resulted from the sum of the Infested Bole Area (IBA) and the Infested Branch Area (IBRA). IBA was computed as:

IBA = diameter x length of the attacked trunk portion, where the diameter value was calculated as the mean of the trunk diameters at the beginning and end of the insect colonisation points.

IBRA of each n-whorl was computed as:

IBRA_n = (diameter x length of the attacked portion of one branch) x number (n) of colonised branches in the whorl, where the diameter value was calculated as the mean of the branch diameters at the beginning and end of the insect colonisation points.

Hence, the TCBS was equal to: $\mathrm{TCBS} = \mathrm{IBA} + \sum^{1}_{n} \mathrm{IBRA}_{n}$.

In order to determine the number of colonizing and emerging beetles, ten branch samples were collected (45 and 5 cm in length and diameter, respectively; N=180) from each of the three felled tree in a spot. Samples were caged outdoors (each group in different plastic pipes) at the university campus in Padova. All *I. acuminatus* adults swarmed during the following spring from each cage (representing a tree), were collected and counted. When emergences ceased, all branches were debarked to record the occurrence of dead or alive beetles remained under the bark. In one branch randomly chosen among the ten in each cage all mating systems were analysed in order to estimate the density of colonising males (*i.e.*, number of nuptial chambers), females (*i.e.*, number of egg galleries) and, consequently, total colonisation density (*i.e.*, number of males and females). One of the analysed mating systems was randomly selected to record the number of galleries of maturation feeding starting from the pupal cells, indicating the number of pre-emerging adults. This value was then compared to the overall amount of beetles emerged from the branches and found under the bark.

The number of beetles collected in each cage (emerging adults plus adults remaining under the bark) and density variables (colonising males and females, galleries of maturation feeding) were divided by the corresponding total bark surface of the branches to obtain mean values per dm^2 of bark.

The overall amount of colonising beetles needed to kill trees in spring 2008 in the treated area was estimated multiplying the mean number of attacking adults recorded from samples of the infested material by the total colonised bark surface (TCBS) of the average-sized tree infested during the spring and by the total number of infested trees. The number of monovoltine first generation and second generation adults overwintering in the infested material was multiplied by the total colonised bark surface (TCBS) of the average-sized tree cut during the treatment and then by the number of infested trees colonised in spring and summer respectively, obtaining the number of potential emerging beetles killed by the suppression cutting.

Statistical analysis

Diameter at breast height, size of the spots and number of beetles caught by traps (dependent variables) were averaged within the year for each location (treated and control areas). Values of all variables recorded from each tree were averaged within the spot (N = 18). Mean values are reported ± standard error, unless otherwise specified. An independent factor (year or generation) was tested by a one-way ANOVA for each variable. To fit the assumptions of the ANOVA, variables were log transformed, if necessary. If the ANOVA assumptions were not met, the Kruskal-Wallis test was performed. Significance was determined at $p<0.05$. Regression analysis was used to test relationships between the number of beetles caught and the number of infested trees. Statistical analyses were performed using the software programs R (version 2.7.0) and Statistica 8 (Statsoft, Inc 1993).

Results

Monitoring of infestation spots

Totally, 176 infestation spots (154 in the treated and 22 in the control area) with 6,082 killed trees (5697 in the treated and 385 in the control area) were recorded in the study area from 2006 to 2009. The size of spots ranged from 2 to 390 killed trees with a maximum radius of about 70 m. Every spring spots were formed in new locations (that is, no old spots continued to grow in the following year). In 2007 and 2008 trees were killed also in the summer (by new generation beetles from the current year) in addition to the trees killed in the spring, while in 2009 no such summer attacks occurred. For 2006 we only have the total number of killed trees but do not know if trees were killed during the summer. All summer-killed trees were found adjacent to spots of trees killed in the spring the same year. Summer-killed trees only occurred in spots with at least 10 trees killed in the spring. Moreover, the number of trees killed during the summer was significantly related to the number of trees killed in the spring in the same spot (Colombari et al., unpublished data).

The number of spots in the whole area slightly decreased from 2006 to 2007 (58 and 53 spots, respectively), whereas the number of recently infested trees doubled in one year (1734 in 2006 and 3475 in 2007). As a result, mean spot size increased significantly in 2007 (65.6 ± 11.0 trees/spot; $F_{(1, 111)} = 12,60$, $p<0,001$) (Fig. 2).

In 2008, the year after the suppression treatment, the number of spots slightly decreased by about 20% in treated area (from 48 to 37 spots: - 22%), whereas in the control it showed a three-fold increase (from 5 to 14 spots: + 280%). Two years after the treatment, in 2009, the number of spots decreased by 62% in treated area (from 37 to 14 spots) and by 100% in control area (from 14 to 0 spots) as the total absence of new infested trees in both late spring and summer.

Considering the mean number of trees per spot, it fell sharply after the cutting in the treated area (from 70.2 ± 12.0 to 12.6 ± 1.7 trees; $F_{(1, 83)} = 17.45$, $p<0.001$) and then remained more or less constant in the following year (2009: 11.0 ± 2 trees). On the contrary, in the untreated area no significant variations were observed in the mean size of spots (from 21.0 ± 2.9 to 18.0 ± 3.8 trees) (Fig. 3). About 50 % of the 2008 spots were small in size (up to 10 trees) in both treated and untreated areas (51.4 and 42.9 %, respectively).

During the infestation diameter and height of attacked trees decreased between years in treated area, showing in 2009 the lowest values ($F_{(3,110)} = 4.30$, $p<0.01$; $F_{(3,110)} = 9.79$, $p<0.0001$ respectively). No differences were observed considering the control area or comparing the two locations.

Monitoring of population densities with traps

Comparing treated and control areas, mean trap catches did not differ in both 2008 and 2009, (treated area: 238.7 ± 32.9 beetles in 2008 and 55.6 ± 28.9 in 2009; control area: 258.0 ± 121.3 in 2008 and 92.3 ± 35.3 in 2009), with only 9 - 29 % of the beetles emerging during the second peak in summer. In the treated area mean catch in the traps slightly differed between 2007 and 2008 (219.5 ± 21.4, N = 65 and 238.7 ± 32.9, N = 12, respectively), reaching in 2009 the lowest value (55.6 ± 28.9, N = 7; $H_{(2, N=84)} = 11.75$, $p<0.005$), whereas in control area there were no differences between 2008 and 2009 (258.0 ± 121.3, N = 3 and 92.3 ± 35.3, N = 3, respectively).

Relating the number of infested trees recorded in both treated and control areas in each season (spring and summer) to the mean number of beetles caught by traps in the season, a positive linear relationship was observed ($R^2 = 0.43$, $F_{(1, 8)} = 6.02$, $p<0.05$, N = 10) (Fig. 4). In addition to the absence of new infested trees in the control area in summer 2008 and throughout the whole 2009, no infested trees were also observed in the treated area in summer 2008, when the lowest number of beetles caught was registered (12.43 ± 12.01).

Estimation of the size of colonizing and hibernating population of beetles in killed trees

The TCBS was significantly related to tree diameter at breast height (TCBS = 0.387 x diameter at breast height − 3.55) ($R^2 = 0.71$, $F_{(1,11)} = 27.51$, $p<0.001$).

In spring 2008, the attacks on the 467 trees in the treated area were conducted by about 5.5 million colonising beetles (mean attack density 24.4 ± 2.9 beetles/dm^2; TCBS = 4.9 m^2, with a mean dbh = 21.8 ± 0.6 cm). Colonisation densities (number of colonising males and females) were similar between generations and years and in 2008 did not differ between areas (treated and control).

The emergence data from branches collected in both June 2007 and 2008, colonised in the spring of the same year, demonstrated that about half of the offspring of the first generation (50.5% and 55.5% in 2007 and 2008, respectively) did not emerge in summer developing a further generation but remained under the bark of the infested material. In 2007 this value corresponded to 0.4 ± 0.2 beetles/dm^2, as the others had already swarmed before collection.

The number of second generation adults effectively found overwintering under the bark of the trees sampled in autumn 2007 (during the treatment) was very low (1.3 ± 0.3 beetles/dm^2), the 4.2% of the number of pre-emerging adults estimated on galleries of maturation feeding (31.1 ± 4.5 beetles/dm^2), suggesting also in this case that large part of the beetles had left the trees before collection. Thus, by cutting all the 2007 infested trees (2475 attacked in spring and 1000 in summer) about 1.1 million monovoltine and bivoltine beetles were killed (TCBS =5.3 m^2, with a mean dbh = 22.8 ± 0.7 cm) corresponding to a 17 % reduction in the 2008 spring damage.

Discussion

Our results show that management strategies of *Ips acuminatus* outbreaks in the south-eastern Alps did not produce the expected results, mainly because the infested trees removed at the end of the summer were already left by a great part of the beetles (96 %) that spent the winter elsewhere. This aspect of the beetle biology, known (Francke-Grossmann, 1963) but poorly explored, had dramatic consequences on the success of the sanitation felling.

As many other bark beetles following the cooperative (or mass attack) strategy, *I. acuminatus* showed the typical spot infestation pattern (Turchin et al., 1998; Lieutier, 2004). Because the beetle is bivoltine at our latitude, the attack behaviour of the insect is seasonally dependent with beetles flying and attacking new host trees in spring and summer (Colombari et al., submitted). New spots are formed in spring, when second generation overwintering beetles disperse and aggregate to form local infestations. In summer, part of the F1 offspring (bivoltine) swarms and attacks the neighboring uninfested trees on the edge of the spot, whereas the others (monovoltine) remain under the bark of the infested trees. Spots remain active no longer than spring of the next year when beetles disperse and new spots can be formed. Thus, before extinguishing, a spot is able to ‘produce’ two beetles generations. During the spring, from a spot formed in the previous year, can emerge beetles of both first (monovoltine) and second generations. However, considering the occurrence of different overwintering sites (Colombari et al., submitted) and the limited spot growth phenomenon (unpublished data), we observed that the total amount of adults swarming from an old spot is very low, thus reducing the possible effects of a suppression treatment. Furthermore, cutting was applied during the epidemic phase of the outbreak, as in 2007 the infestation had grown in number and size, surpassing the stand-mesoscale eruptive threshold (Raffa et al., 2008) and with successful mass attacks against large diameter trees. It is already known that a great and repeated effort occurs to reduce an ongoing beetle outbreak (Safranyk, 2004), but in our study we assessed that suppression cutting is not cost-effective to control *I. acuminatus* population.

As measures of treatment effectiveness, we can consider the ‘post-treatment tree mortality’ (Foltz, 1979) and the new spot occurrence (proliferation) in controlled and uncontrolled infestations (Billings and Pase, 1979). The strong reduction or absence of further tree mortality and number of new spots after the cutting may indicate if the treatment was effective (Foltz, 1979): if beetles disperse from an old spot to initiate new infestations (proliferation) nearby, in the vicinity of spots controlled by any kind of method able to reduce the number of beetles, it would be expected a lower number of new spots than that recorded near spots without treatment (Billings and Pase, 1979). Therefore, assuming that control treatment was the only factor which significantly influenced infestation trend, the treatment could be considered successful, as both spot size and proliferation were significantly affected by the treatment two years after suppression. We observed, however, that the number of spots did not vary greatly after the cutting (48 spots in 2007 and 37 in 2008). This effect was probably due to the large part of beetles, of both first and second generations, that overwinter in sites different from the infested bark where they have developed, as observed during a study conducted on *I. acuminatus* biology (Colombari et al., submitted). Leaving the trees in late summer, beetles avoid the negative consequences of the treatment on both population level and dispersion during the following spring, as demonstrated by mean trap catches the year after the treatment (219.4 ± 21.4 in 2007 and 238.7 ± 32.9 in 2008). This value, moreover, did not differ from that recorded in uncontrolled area. Further, we estimated that only the 17 % of the potential attacking beetles were killed, avoiding attacks on about one hundred trees, number too low to justify by itself the sharp decrease in mean size of the spots (1/5 ca) after the cutting.

Without treatment, the spots were expected to grow both in size and number, as actually happened in the control area: but an unexpected result was the total absence of new infested trees in 2009. As the control area was under the same weather conditions acting in the treated one, we can exclude that only climatic conditions limited bark beetle success. We can hypothesize three possible explanations for this collapse: bark beetle migration, depletion of resources and incidence of density-dependent factors. Maximum distance between old and new spots in the valley ranged from 11 to about 12.5 km: it is not improbable that *I. acuminatus* could disperse up to this distance, as *I. sexdentatus* can fly for more than 20 km (Jactel and Gaillard, 1991) and *I. typographus* for 45 (Byers et al., 1989), but we have no evidence of this. Migration might be also a consequence of the absence of suitable hosts, but the lack of persistence of beetles and movement to new areas were previously assessed for other bark beetles despite the presence of acceptable hosts (Mitchell and Preisler, 1991; Borden, 1993; Robertson et al., 2007). Nevertheless, the absence of suitable hosts is known to be an important factor in population dynamics, following the resource-depletion model recently developed for forest insects outbreaks by Økland and Bjørnstad (2006). When a high number of susceptible host trees is available, beetle population may increase above the threshold for colonizing susceptible trees and even beyond the outbreak threshold. As a consequence of the large number of attacking beetles, susceptible trees are rapidly depleted and population declines below the threshold of tree colonization as subsequent generations of beetles breed in trees of reduced nutritional quality or increased resistance, suffering high mortalities (Safranyk, 2004; Økland and Bjørnstad, 2006). Furthermore, different population densities entail a different incidence of density-dependent mortality factors, such as natural enemies, that may have an important role in population regulation together with other factors, such as intraspecific competition (Kenis, 2004). Lastly, another important aspect we have to consider is the high percentage of small spots (43%) in 2008 in the control area. In previous studies the susceptibility of small spot to extinction is described and we observed that there were not summer-killed trees adjacent to spots with up to 10 trees killed in the spring (Colombari et al., unpublished data). Authors report the phenomenon is presumably influenced by natural enemies (Reeve, 1997) and by the difficulty that small populations have in overwhelming tree defenses (Berryman et al., 1985; Ayres et al., in press). Considering that also in the treated area about the 50% of the 2008 spots had up to 10 trees, the decline of infestation observed in 2009 might be explained following this hypothesis.

Many, if not all, the mentioned hypotheses might be reasonably accepted in explaining the evolution of the outbreak. Undoubtedly, the treatment leads to a reduction in the number of colonising beetles, but considering the cost in time and effort, the result is not satisfactory as confirmed by the indicative values we estimated. This is mostly due to the occurrence of different overwintering sites reached by both first and second generation adults. Moreover, the absence of any kind of symptom other than change in colour of the crowns, evident late in the season, makes it impossible to identify the infested trees before beetles leave them and to apply the cutting in the proper time, either in spring or in summer. For the same reason, the interesting positive relationship found between trap catches and damage level in the year can not help management decision making: in addition, similar trap catches often do not correspond to similar damage levels, as the possible absence of suitable hosts or incidence of other factors before and during dispersal and colonisation phases.

Considering possible alternative control strategies, the low total amount of beetles caught that we obtained by using pheromone traps leads to exclude the hypothesis of mass trapping (14000 beetles caught by 65 traps in 2007). These data, however, greatly differ from those recently collected in Spain where more than 10,000 *I. acuminatus* adults entered each Lindgren funnel model trap during the monitoring season (Pérez and Sierra, 2006). Moreover, during a four years period (2002-2005), different pheromone aggregative lures were tested, obtaining similar results (Hernandez et al., 2004, 2007). Considering that the Multi Funnel trap used in our experiment is very similar to that used in Spain, the considerable difference in attractiveness between our and Spanish results could be explained with the low efficacy of the pheromone lure we used. By improving the quality of the lure, mass trapping seems feasible, although the rapid appearance and evolution of bark beetle infestations and the clumped distribution of susceptible hosts related to the mosaic of conditions that contribute to susceptibility (Coulson, 1979) add difficulties in decision making of forest policy managers. For all these reasons, the control of *I. acuminatus* outbreaks seems to be very difficult. As this species is becoming a new threat to the alpine stands of Scots pine (Wermelinger et al., 2008; Colombari et al., submitted), more work is required in order to define the most appropriate control practice.

Acknowledgments

We are grateful to Valerio Finozzi and Edi D'Ambros (Regional Forest Services) for helping the surveys and the trapping and providing precise data on the cutting. A special thanks to Attilio and Gino, for the precious practical help in all field activities. We also greatly appreciate the logistic support in sampling activities given by Tommaso Anfodillo, Fausto Fontanella, Roberto Menardi of the Centro Studi per l'Ambiente Alpino 'Lucio Susmel' of the University of Padova in San Vito di Cadore. Lastly, we thank Edoardo Petrucco Toffolo and Lorenzo Marini for the constructive comments on the manuscript.

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Fig 1 Map of spot distribution in 2007: grey area corresponds to the control area excluded from the treatment.

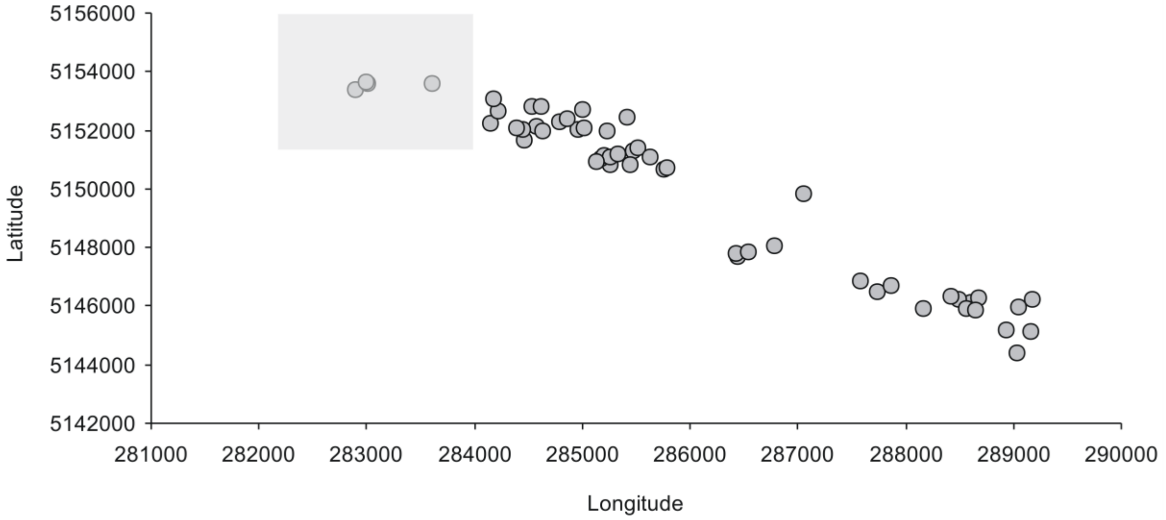


Fig 2 Number of spots and mean size of the spots (± SEM) before the treatment.

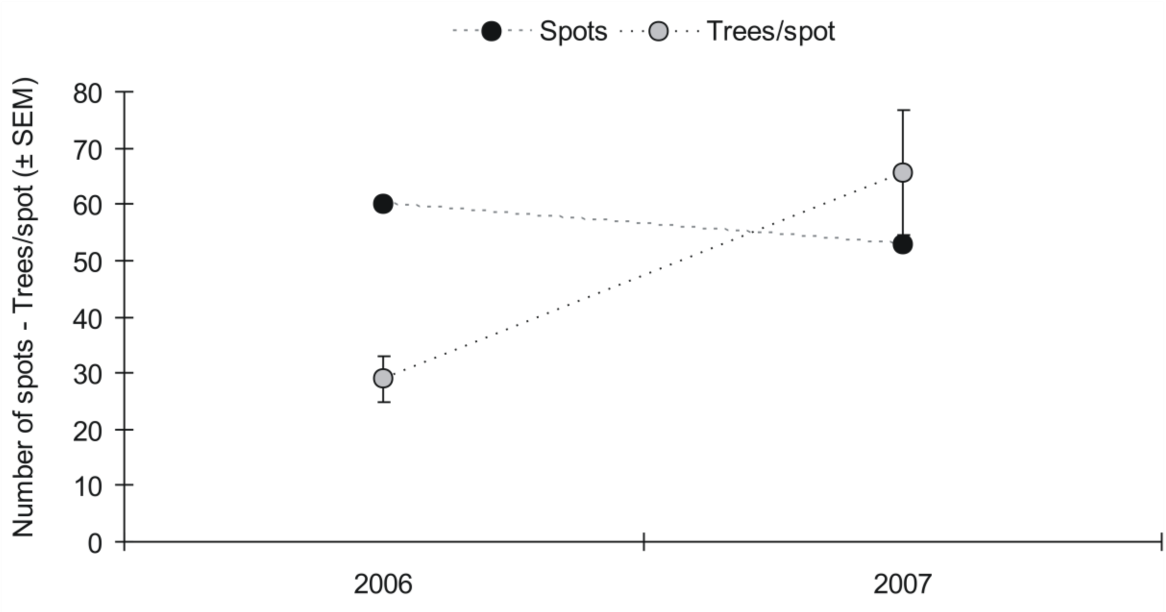


Fig 3 Mean size of the spots (± SEM), before and after the treatment, in both treated and control areas.

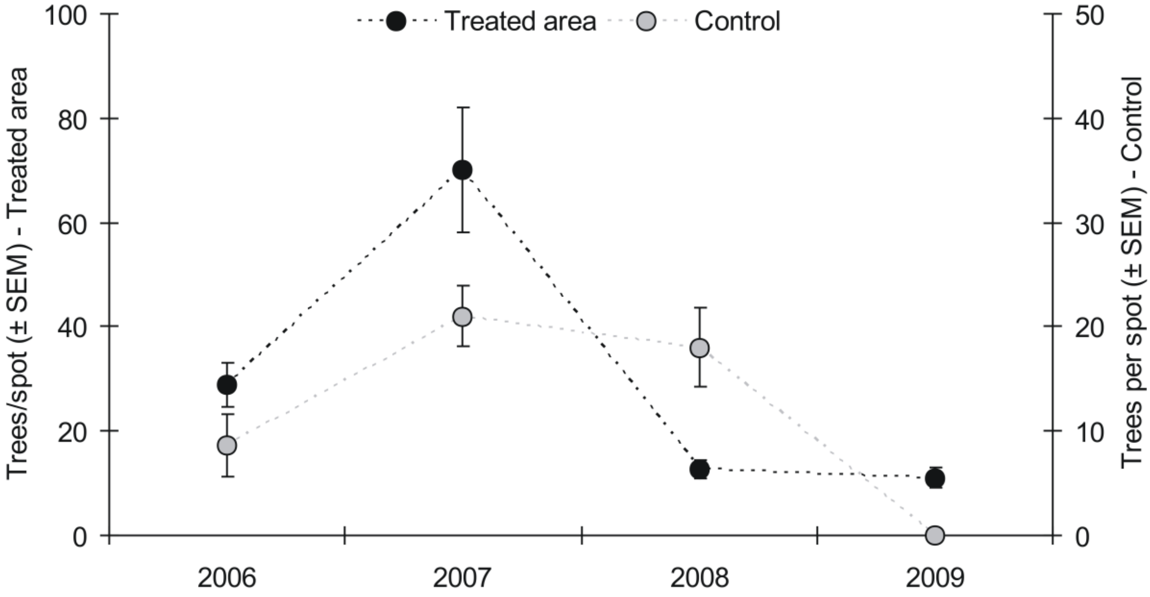
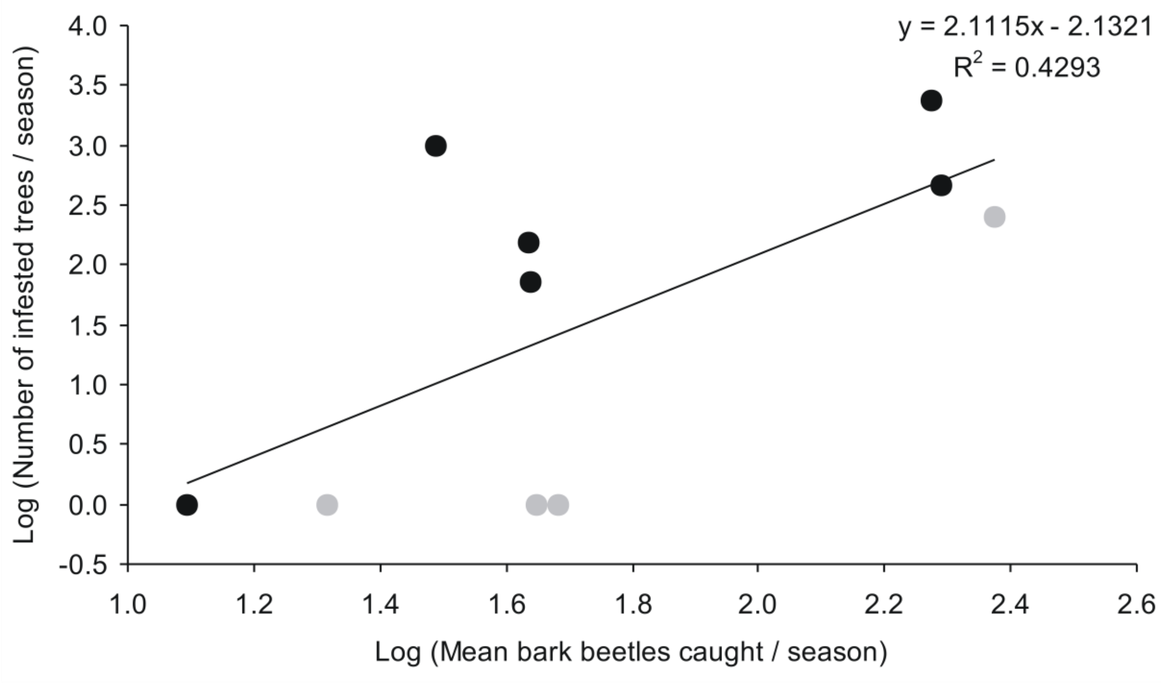
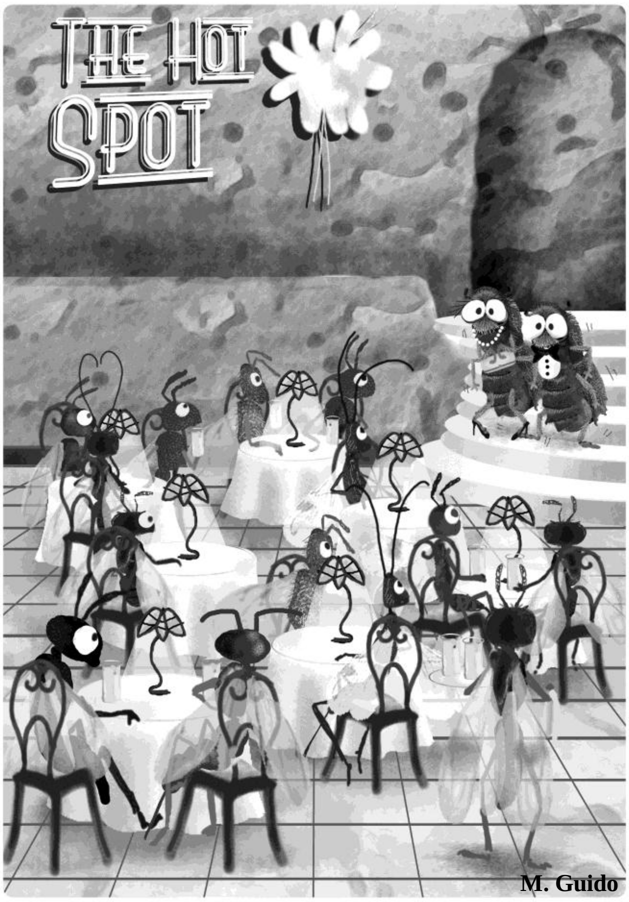


Fig 4 Relationship between mean number of beetles caught and number of infested trees in each season (spring and summer) in both treated (TA - ●) and control (CA - ●) areas (N = 10).



Chapter IV

Natural enemies associated with **Ips acuminatus** *in the south-eastern Alps* (*)



(*) This paper has not reached yet the final phase of preparation and some analyses are still going on. It is included here, however, to give a general, although preliminary, idea of the work carried out on the natural enemies of *Ips acuminatus*.

Colombari F., Battisti A., Faccoli M.

Natural enemies associated with Ips acuminatus *in the south-eastern Alps*

I collected most of the data, analysed them and drafted the manuscript.

Abstract

- Natural enemies of *Ips acuminatus* might have an important influence on population dynamics and on the pattern of spot development. In the south-eastern Alps, infested branches collected during three years of an outbreak lead to assess the natural enemies complex of the beetle.

- Six species of predators belonging to Diptera (Stratiomyiidae: *Zabrachia* spp.; Dolichopodidae: *Medetera* spp.) and Coleoptera orders (Tenebrionidae: *Corticeus linearis*; Rhizophagidae: *Rhizophagus* spp.; one species of Staphylinidae and one of Nitidulidae families) were found: overall predator density was inversely related to the abundance of bark beetles. With regard to parasitoids, eighteen hymenopteran species were collected, represented by Pteromalidae (72.2 %), Braconidae (17.5 %), Eurytomidae (9.7 %) and Eupelmidae (0.6%). *Metacolus unifasciatus*, *Dendrosoter hartigii*, *Rhopalicus quadratus*, *Roptrocerus xylophagorum*, *Tomicobia acuminati*, *Dinotiscus colon* and *Eurytoma arctica* were the most common species. All species overwinter in the host tree. Except for *Tomicobia acuminati*, an adult endoparasitoid, they are all larval and pupal ectoparasitoids.

- Action of the enemy complex was related to the phase of the outbreak: overall parasitism by larval and pupal parasitoids, as well as for the adult parasitoid *Tomicobia acuminati*, was density-dependent, even if density of each species responded differently to the change of host density or to the generation.

- Parasitoids and predators are known to be factors that may restrict the spatial spread of an insect outbreak. We hypothesize that natural enemies contribute to the extinction of spots especially when beetle populations are reduced in size.

Keywords: engraver beetle, natural enemies, parasitoids, predators, population dynamics**.**

Introduction

Main natural mortality factors acting on scolytid bark beetle (Coleoptera: Curculionidae, Scolytinae) populations are of great interest, as this group includes some of the most damaging insects attacking conifers worldwide. The economical importance of these forest insects and the recent attention to sustainable forest management, led to consider all the possible natural mortality factors, notably insect parasitoids and predators (Kenis et al., 2004).

For many years, enemies have been considered a secondary mortality factor in bark beetle dynamics, whereas fluctuations of bark beetle populations have been mainly attributed to factors such as resistance of the host tree (Reeve, 1997). But studies on the regulatory capacity of antagonists revealed they could be important in bark beetle dynamics (Reeve, 1997), maintaining bark beetle population below the epidemic threshold, singly or in interaction with other mortality factors, for instance interspecific and intraspecific competition (Moeck & Safranyk, 1986).

Natural enemies of bark beetles comprehend a wide variety of predators and parasitoids feeding on beetles on the outer bark and within trees. In particular subcortical predators and larval ectoparasitoids may have different responses to different host population densities, varying their regulatory power in epidemic or endemic situations (Beaver, 1967). Overall, impact of antagonists can reduce by a large amount the number of emerging beetles, up to 80 % (Weslien, 1992; Wermelinger, 2002).

The main aims of the study, carried out in the Dolomites (south-eastern Alps) during five consecutive beetle generations, were to assess the enemy complex associated with *I. acuminatus* under the bark, to quantify the impact of antagonists on breeding performance and to clarify the role of predators and parasitoids in the population dynamics of *I. acuminatus*.

Materials and methods

The Study Area

Field data were collected in a mature (>100 years) Scots pine (*Pinus sylvestris*) forest located in the Dolomites (NE Italian Alps) (46°27’ N, 12°12’ E, about 1000 m a.s.l.) where an outbreak of the pine engraver beetle *I. acuminatus* (Gyllenhal) (Coleoptera: Curculionidae, Scolytinae) has been active since 2005 (Faccoli et al., 2007; Colombari et al., 2008). The terrain is mountainous, marked by steeples and pinnacles, with vertical walls and sheer cliffs separated by narrow and deep valleys. The valley is characterized by a continental climate, displaying cold and rather dry winters and mild and wet summers. In the last 30 years, the mean annual precipitation at Col de La Roa (1,100 m a.s.l.), the nearest meteorological station, is 1100 mm, with maximum in July. Mean annual temperature is 7.2°C and extremes vary from - 11°C to + 29°C. The coldest and warmest months are January and July, respectively. Scots pine is the dominant tree species, which covers most of the forested land, followed by Norway spruce (*Picea abies*) and dwarf mountain pine (*Pinus mugo* subsp. *mugo*). Scots pine stands are located on south-western facing slopes and have a mean density of about 300 trees per hectare.

Natural enemy and bark beetle populations under the bark

Monitoring of the infestations was carried out twice a year (from autumn 2006 to autumn 2009) by detecting infestation spots recently formed and counting the number of infested trees in each spot. An overall amount of thirty-five spots of trees infested by *I. acuminatus* were randomly selected and sampled for three consecutive years in April (six spots in 2007, 2008 and 2009) and June (six spots in 2007, eleven in 2008), as soon as pine crowns faded after the beetle attacks conducted in spring and summer by hibernating and new generation beetles, respectively (Colombari et al., submitted). As a sanitation felling was applied in autumn 2007 in order to limit damage and prevent further expansion of the beetle population, in the treated area the sampling scheduled for spring 2008 was carried out during the cutting (October 2007) (Colombari et al., unpublished data). Sampling was standardized by selecting, in each spot, three infested trees (N = 105) and by collecting from each tree ten branch samples (45 and 5 cm in length and diameter, respectively; N = 1050). Samples were transported at the university campus in Padova in the same day and held in outdoor cages (each group of branches, representing a tree, in a different plastic pipe). Once a week, cages were checked and all emerged insects were collected, counted and sexed. Parasitoids were determined to species and identification was confirmed by a specialist. Predators were determined to genus or family.

At the end of the emergence, all branches were debarked and dead or alive insects found under the bark were recorded. On one branch per cage (*i.e.* per tree, N = 105) randomly chosen, all *I. acuminatus* mating systems were analysed in order to estimate the density of colonising males (*i.e.*, number of nuptial chambers), females (*i.e.*, number of egg galleries) and the total colonisation density (*i.e.*, number of males and females). Furthermore, in 2007 and 2008, on one mating system per branch the length of egg galleries and the number of egg niches, larval galleries, pupal cells and maturation galleries of callow adults were also recorded (Colombari et al., submitted). All variables were reported as mean values per dm^2 of bark.

Impact of natural enemies on **I. acuminatus** *population (2007 and 2008)*

Predation was assessed by multiplying the number of predators by their consumption rates observed in previous studies. Among predators, the dipterous *Medetera* and *Lonchaea* spp. are known to prey 6 bark beetle larvae during their development, whereas the beetles *Rhizophagus* and *Thanasimus* spp. kill 15 and 47 preys, respectively (Herard and Mercadier, 1996; Kenis et al., 2004). We assumed a mean consumption rate of 4 larvae (one of the lowest values indicated in Kenis et al., 2004) for the other species. This value was probably underestimated, but might be compensated by the occurrence of cannibalistic and generalist predators, which reduced the impact of the complex of antagonists on the beetle population (Kenis et al., 2004). Parasitoids usually develop from a single host larva. Therefore, the number of killed hosts was equal to the number of parasitoids. Hyperparasitoids and adult parasitoids were excluded from the analysis as they do not contribute to *I. acuminatus* preimaginal mortality.

The sum of killed beetles and galleries of maturation feeding (*i.e. I. acuminatus* adults actually emerged) was considered as the potential emerging population in the absence of antagonists. On this value, mortality percentages due to both predators and parasitoids were estimated in each 2007 and 2008 generations.

Statistical analysis

Values of all variables recorded from each tree were averaged within the spot (N = 35). All mean values were reported ± Standard Error of the Mean (SEM), unless otherwise specified. One independent factors, generation_year, were tested by a one-way ANOVA for each variable. To fit the assumptions of the ANOVA, variables were log or arc-sin transformed, if necessary. Significance was determined at $p<0.05$. Regression analysis was used to test relationships between pair of continuous variables. Statistical analyses were performed using the software programs R (version 2.7.0) and Statistica 8 (Statsoft, Inc 1993).

Results

Natural enemy population under the bark

In these three years, a total amount of 16,628 individuals, besides *I. acuminatus,* emerged from the infested branches: 14,354 parasitoids, 2,215 predators and 59 bark beetles (Tab. 1).

Eighteen species of adult hymenopteran parasitoids were identified (Tab. 1) representing for the most part Pteromalidae (72.2 %), followed by Braconidae (17.5 %), Eurytomidae (9.7 %) and Eupelmidae (0.6 %). With the exception of few species rarely collected (4 specimens in three years) (Tab. 1), the others were found in both host generations of each year. The most abundant were *Metacolus unifasciatus, Dendrosoter hartigii, Roptrocerus xylophagorum, Rhopalicus quadratus, Dinotiscus colon, Tomicobia acuminati* and *Eurytoma arctica* (Fig. 1). These parasitoids are poliphagous larval ectoparasitoids, except for *T. acuminati* which is an endoparasitoid on *I. acuminatus* adults (Fig. 2), and *Eupelmus urozonus* and *Mesoplobus typographi*, which are known as hyperparasitoids.

The parasitoid complex reached its lowest density in 2009 ($F_{(4,\ 30)}= 4.41$, $p<0.01$), whereas *T. acuminati* peaked in 2007 (Tab.1). Both densities followed the trend observed in the infested trees (Fig. 3 and Fig. 4). Among the seven most abundant species, only *M. unifasciatus* and *R. xylophagorum* did not vary between years and generations (Tab. 1). *D. hartigii* and *R. quadratus* were more abundant in the first than in the second host generation, whereas *D. colon* and *E. arctica* showed the opposite trend. Both these species decreased in the declining phase of the outbreak (Tab 1). The density of hyperparasitoids was positively related to the density of other parasitoids ($R^2 = 0.42$, $F_{(1, 33)} = 26.10$, $p < 0.001$).

Predators belonged to Diptera, Coleoptera and Neuroptera orders and the most common species were *C. linearis*, *Medetera*, *Rhizophagus* and *Epurea* spp. (Tab. 1). Only the larvae of *Thanasimus formicarius, Zabrachia* and *Raphidia* spp. were collected, whereas in all the other species both larvae and adults were found. Predator density sharply increased at the end of the outbreak, peaking in 2009 ($F_{(4, 30)}= 12.84$, $p<0.00001$) (Fig. 5), as *Rhizophagus* and *Epurea* spp. reached the highest densities during the first brood 2008 whereas *C. linearis* and *Medetera* spp during the second brood 2009 (Tab. 1).

Impact of natural enemies on **I. acuminatus** *population (2007 and 2008)*

The impact of natural enemies varyed according to species abundance and host densities, killing from 4.4 up to 47.6 % of the scolytid population.

I. acuminatus first brood developing in spring always suffered the highest mortality in both 2007 and 2008 ($F_{(3, 25)} = 17.32$, $p < 0.00001$) (Fig. 6), as the overall antagonist impact was inversely related to the host density ($R^2 = 0.22$, $F_{(1, 27)} = 7.68$, $p < 0.01$) (Fig. 7), lower in the first than in the second brood ($F_{(1, 27)} = 11.16$, $p < 0.005$). In detail, mortality due to parasitoids ranged from 1.6 to 25.9%. With respect to the first generation, it was higher in 2007 (16.0 ± 3.1) than in 2008 (8.9 ± 1.9 %) ($F_{(3, 25)} = 3.84$, $p < 0.05$) (Fig. 8) whereas it was similar in the second host-generation (6.1 ± 1.5 % and 7.0 ± 1.0 % in 2007 and 2008, respectively). *T. acuminati* was positively related to the number of dead adults found under the bark ($R^2 = 0.21$, $F_{(1,27)} = 7.07$, $p < 0.05$). The reduction in the parasitoid complex density due to *E. urozonus* and *M. typographi* was very low (up to 0.04 %).

Predation ranged from 1 to 46.7%. It was higher in 2008 (29.2 ± 6.2 %) than in 2007 first host-generation (12.9 ± 0.3) ($F_{(3,25)} = 17.29$, $p < 0.00001$) but similar between years considering the second host-generation (3.1 ± 0.4 and 5.2 ± 1.3, in 2007 and 2008, respectively) (Fig. 9).

Discussion

Our preliminary results reveal that the enemy complex associated with *I. acuminatus* seem to be as rich (18 species) as observed by Herard and Mercadier (1996)(33 species, perhaps not all of them associated with the beetle as there were other species in the logs used for the analysis). In our study, the antagonists were surely associated with *I. acuminatus*, as a very low number of other bark beetle species (59 specimens) emerged from all the sampled branches.

As assessed in a previous study, preimaginal mortality strongly reduced the number of emerging individuals mainly in the first generation, as about 80% of the individuals died before maturation feeding as adults in the bark (Colombari et al., submitted). Large part of the observed mortality is due to overcrowding at the breeding sites. As a consequence of the mass attack strategy, overcrowding of colonising parent beetles is known to be associated with an increasing intraspecific competition for space and mortality (Beaver, 1974; Kirkendall, 1989), resulting in an extremely reduced breeding performance that is negatively related to colonisation density (Colombari et al., submitted). New findings, however, lead to consider the important role that natural enemies may have in determining pre-emergence mortality as they can reduce up to 50 % the number of emerging beetles, with a strongest impact on the first brood developing in spring. However, it is difficult to separate the effect of mortality by natural enemies from the other factors, such as intraspecific competition.

At the large scale, density of antagonists followed two different trends: while parasitoids showed an immediate direct response to the fluctuations of the beetle population, predators had a delayed and inverse response that may play an important role in determining spot size and extinction, especially at the end of the outbreak. The high pressure on first generation exerted by the complex reduce the number of beetles that swarm in summer, involved in the enlargement of the size of the spots (Colombari et. al, unpublished data). The increase in the activity of predators mainly at the end of the outbreak, may explain the occurrence of a large number of small spot more susceptible to extinction. As found by Beaver (1967), it seems that the parasitoids ‘compensate for the loss of regulatory power of the predators at high densities’.

Acknowledgments

We express our gratitude to Marc Kenis, who confirmed the identification of some parasitoid species. We thank the foresters of the Regional Forest Service of the Belluno district for their precious assistance in field work. We also greatly appreciate the logistic support in sampling activities given by Tommaso Anfodillo, Fausto Fontanella, Roberto Menardi of the Centro Studi per l'Ambiente Alpino 'Lucio Susmel' of the University of Padova in San Vito di Cadore.

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Tab. 1 Species composition, total number and relative abundance of parasitoids and predators (percentage of each species calculated on the overall amount of antagonist per dm^2 of infested bark) in *I. acuminatus* generations during the three surveyed years. The category ‘Other species' includes species rarely collected such as *Ecphylus hylesini* ♦ (Ratzeburg, 1852), *Ropalophorus claviventris* ●, *Spathius* Nees von Esenbeck, 1818 and *Microtonus* spp (Braconidae).

● = species not recorded in Italy. ♦ = species not recorded in northern Italy (CK2000 - Checklist of the Italian fauna).

p-value indicates if species density varied with generation_year (one-way ANOVA; N = 35).

Species		**Total number**	**F2 2006/2007**	**F1 2007**	**F2 2007/2008**	**F1 2008**	**F2 2008/2009**	***p-value***
Parasitoids								
Pteromalidae (Hymenoptera)								
Metacolus unifasciatus Förster, 1856		3,558	23.83	21.10	25.90	15.01	11.38	ns
Rhopalicus quadratus (Ratzeburg, 1844)	●	1,641	4.21	21.88	3.61	18.62	2.30	< 0.05
Roptrocerus xylophagorum (Ratzeburg, 1844)		1,787	8.75	11.11	10.33	14.81	6.13	ns
Dinotiscus colon (Linnaeus, 1758)	●	1,620	11.43	5.76	15.09	3.20	8.22	< 0.05
Tomicobia acuminati Hedqvist, 1959	●	1,448	9.40	13.13	8.20	5.97	6.96	< 0.05
Heydenia pretiosa Foerster, 1856	♦	160	2.31	0.32	1.37	0.23	0.10	
Mesoplobus typographi	●	146	0.97	0.46	0.79	1.14	0.17	
Eupelmidae (Hymenoptera)								
Eupelmus urozonus Dalman, 1820		80	0.96	0.17	0.67	0.05	0.49	
Eurytomidae								
Eurytoma arctica Thomson, 1876	♦	1,398	12.10	3.40	13.05	1.85	4.01	< 0.05
Braconidae (Hymenoptera)								
Dendrosoter hartigii (Ratzeburg, 1848)	♦	2,330	16.37	18.27	10.75	17.98	4.17	< 0.05
Dendrosoter middendorffi (Ratzeburg, 1848)	♦	102	1.16	0.05	0.86	0.10	1.10	

Tab. 1 (cont.)

Species	**Total number**	**F2 2006/2007**	**F1 2007**	**F2 2007/2008**	**F1 2008**	**F2 2008/2009**	***p-value***
Blacus humilis (Nees von Esenbeck, 1812)	27	0.44	0.00	0.23	0.00	0.00	
Coeloides bostrichorum Giraud ●	11	0.04	0.00	0.11	0.00	0.22	
Other species	46	0.38	0.07	0.03	0.18	0.13	
Predators							
Cleridae (Coleoptera)							
Thanasimus formicarius (Linnaeus, 1758)	69	0.00	0.76	0.07	1.02	0.60	
Nitidulidae (Coleoptera)							
Epurea spp. Erichson, 1843	385	1.04	1.28	1.51	6.13	1.05	< 0.05
Rhizophagidae (Coleoptera)							
Rhizophagus spp Herbst, 1793	501	0.69	1.66	0.49	11.52	0.74	< 0.05
Staphylinidae spp. (Coleoptera)	47	0.21	0.06	0.37	0.28	1.23	
Tenebrionidae (Coleoptera)							
Corticeus (*Hypophloeus*) *linearis* Fabricius, 1790	393	1.69	0.24	2.87	1.09	10.36	< 0.05
Dolichopodidae (Diptera)							
Medetera spp. Fischer v. Waldheim, 1819	703	3.71	0.02	3.01	0.08	37.04	< 0.05
Lonchaeidae (Diptera)							
Lonchaea spp. Fallén, 1820	37	0.00	0.05	0.07	0.29	3.33	
Stratiomyiidae (Diptera)							
Zabrachia spp. Coquillett, 1901	23	0.00	0.00	0.08	0.46	0.04	
Raphidiidae (Neuroptera)							
Raphidia spp. Linné, 1758	57	0.31	0.22	0.54	0.00	0.24	
Total number	**16,569**						

ns = not significant

Fig. 1 The most abundant species. *Metacolus unifasciatus* (a), *Dendrosoter hartigii* (b), *Roptrocerus xylophagorum* (c), *Rhopalicus quadratus* (d), *Dinotiscus colon* (e), *Tomicobia acuminati* (f) and *Eurytoma arctica* (g).

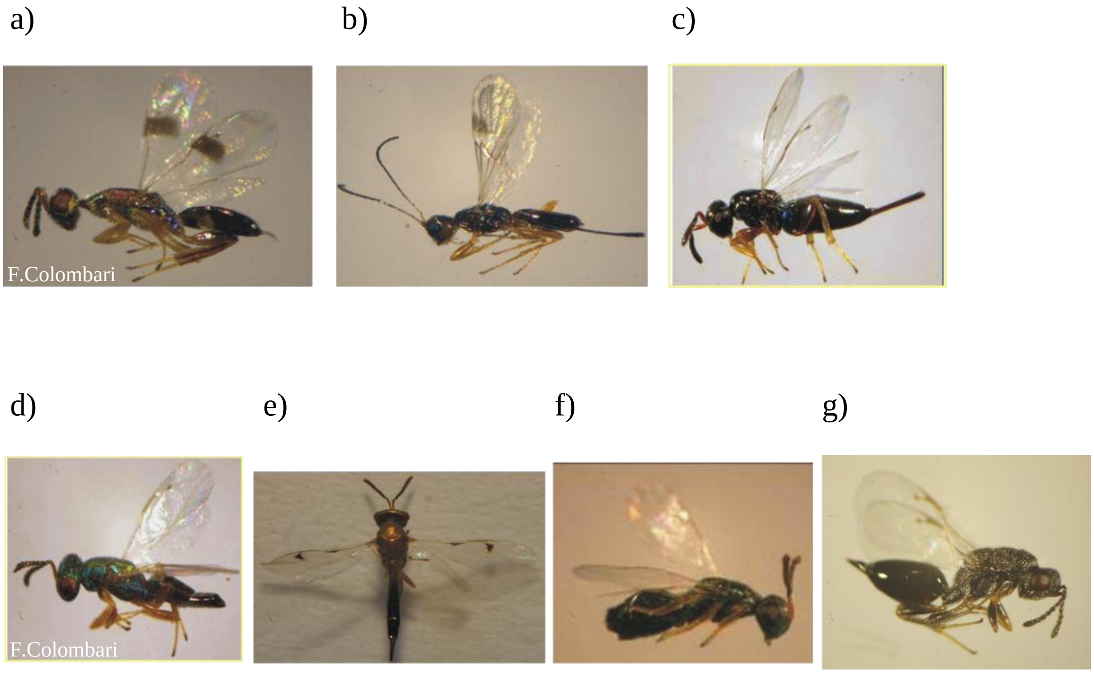

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Fig. 2 *Tomicobia acuminati*, endoparasitoid on *I. acuminatus* adults.

Fig. 3 Trend of the infestation (number of killed trees) and parasitoids density per dm^2 of bark (± SEM; N = 35) across three years and five generations.

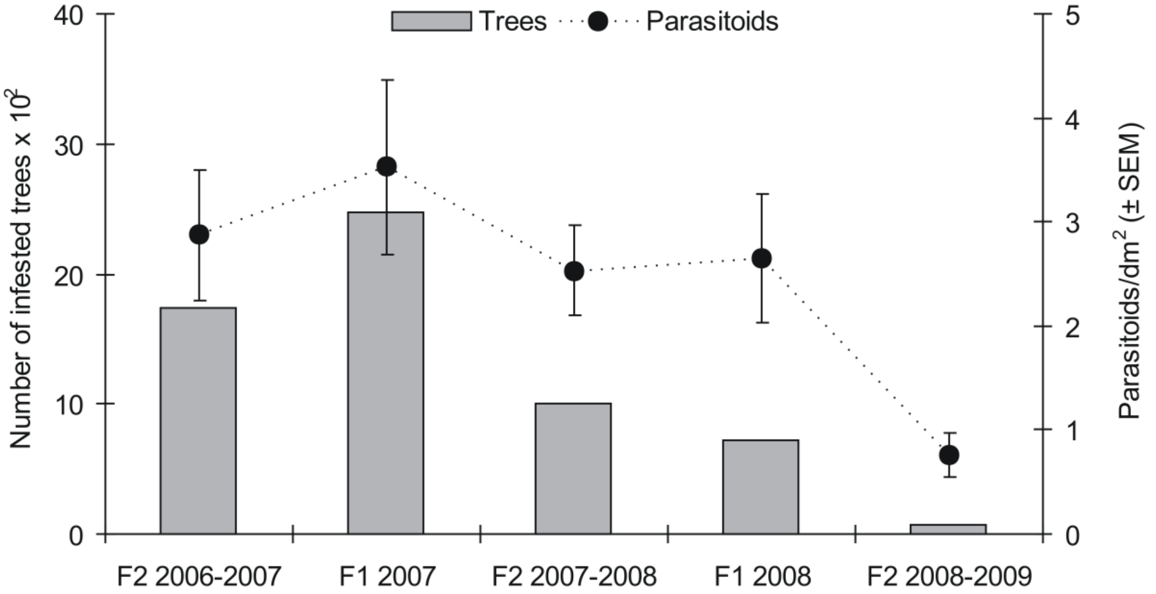


Fig. 4 Trend of the infestation (number of killed trees) and *Tomicobia acuminati* density per dm^2 of bark (± SEM; N = 35) across three years and five generations.

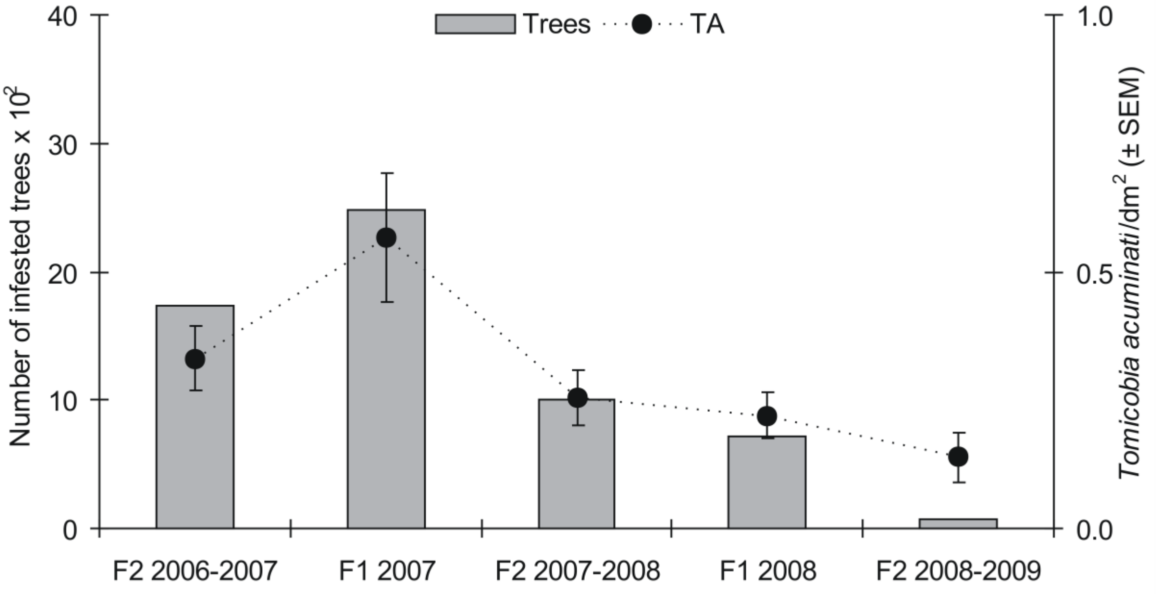


Fig. 5 Trend of the infestation (number of killed trees) and predators density per dm^2 of bark (± SEM; N = 35) across three years and five generations.

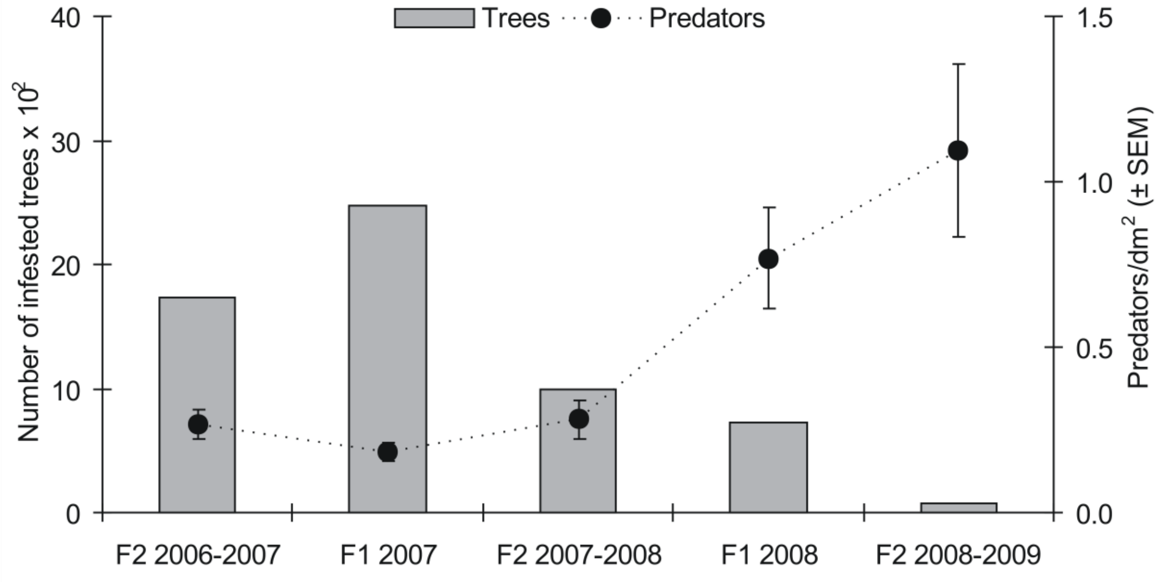


Fig. 6 Natural enemy impact (percentage of bark beetle mortality ± SEM; N = 29) and trend of the infestation (number of killed trees) in 2007 and 2008.

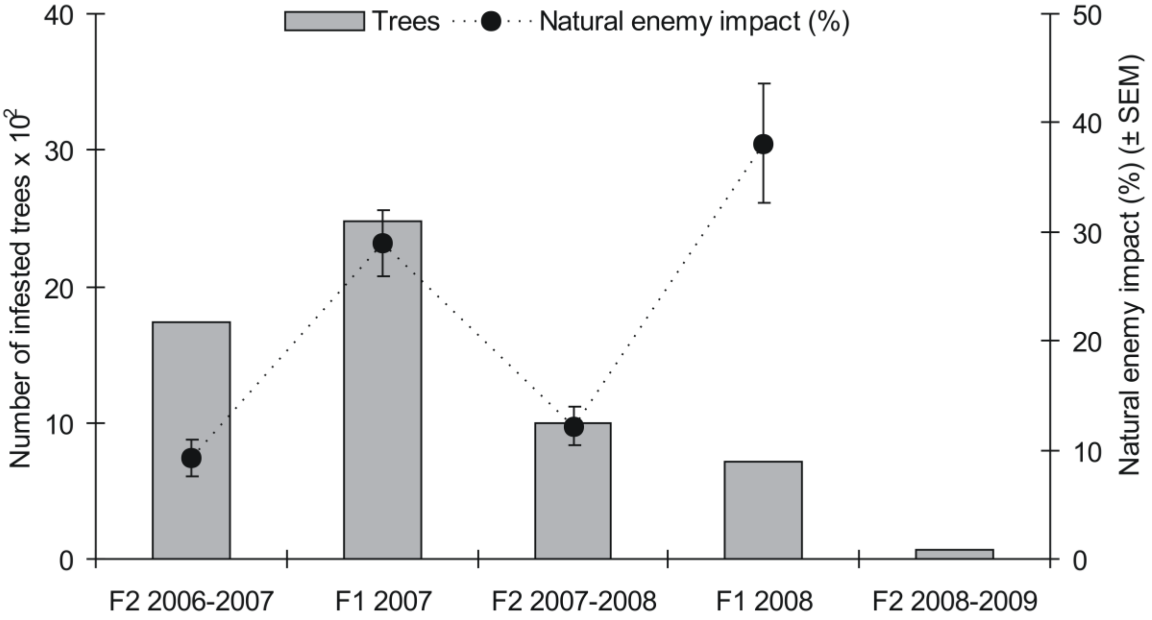


Fig. 7 Relationships between natural enemy impact (percentage of bark beetle mortality) and host density (potential emerging *I. acuminatus* adults per dm^2 of bark) in 2007 and 2008 (N = 29).

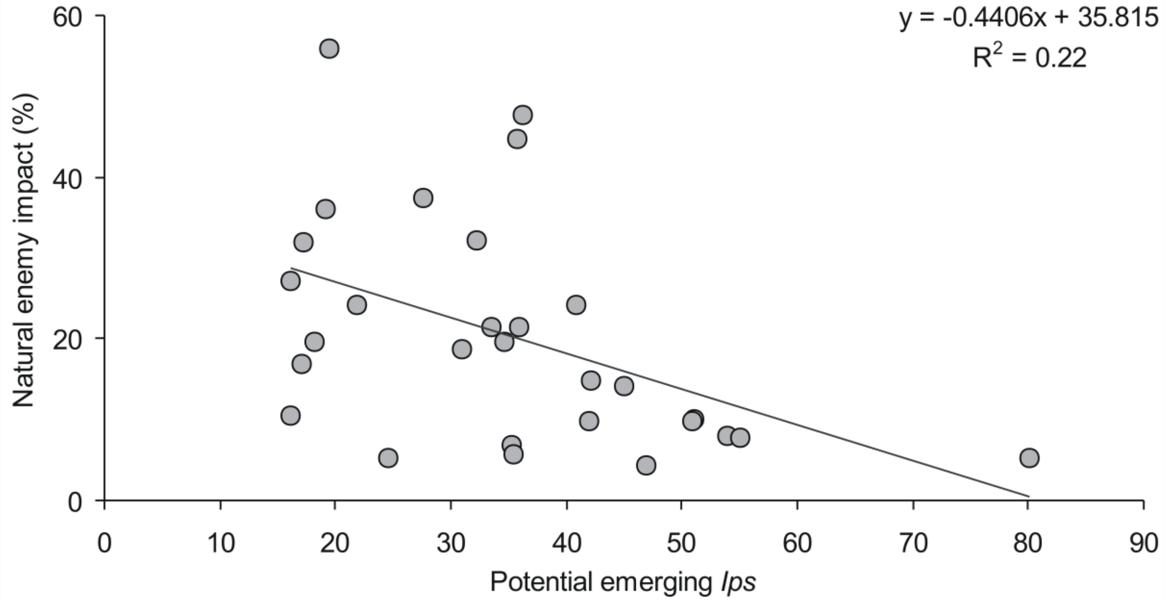


Fig. 8 Parasitoid impact (percentage of bark beetle mortality ± SEM; N = 29) and trend of the infestation (number of killed trees) in 2007 and 2008.

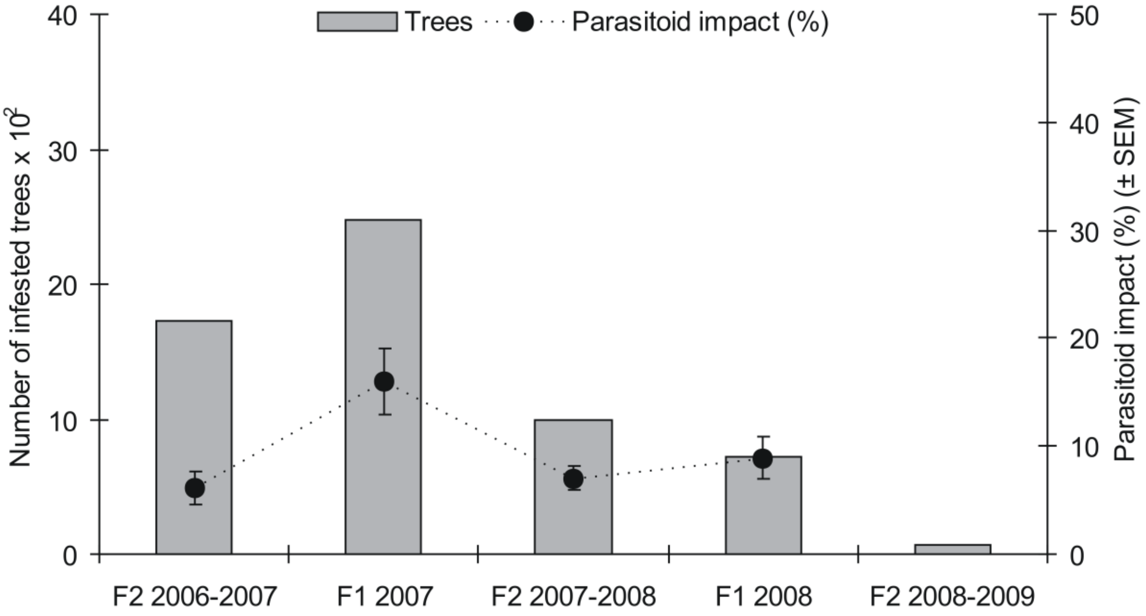
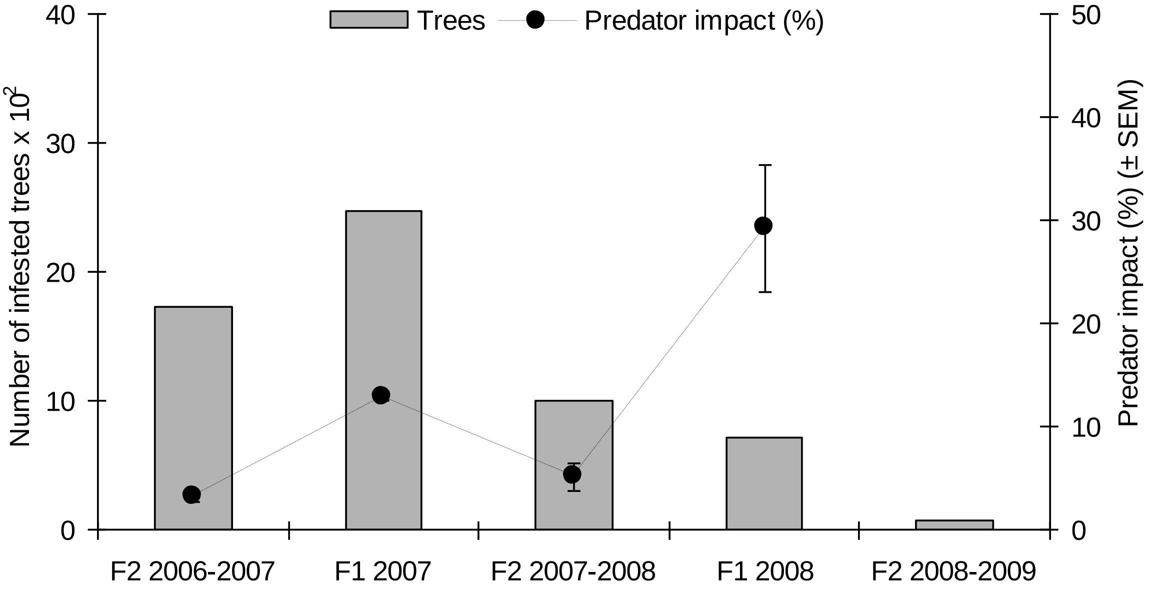


Fig. 9 Predator impact (percentage of bark beetle mortality ± SEM; N = 29) and trend of the infestation (number of killed trees) in 2007 and 2008.



Conclusions

The occurrence of the outbreak of *Ips acuminatus* in the Dolomites (south-eastern Italian Alps) offered the unique opportunity to study both natural history and population dynamics and to generalise the results in the context of a larger geographic scale in the Alps. This is important because the beetle plays a role in the progressive Scots pine dieback possibly related to global warming. In these last years an increasing mortality was registered in Italy (Aosta Valley) and Switzerland, as well as in Spain. Despite knowledge on mechanisms underlying tree dieback is still limited, climate seems to be an important variable, leading to hypothesise that climate change may be a direct or indirect cause, either increasing pest performance or reducing tree resistance. High temperature spells and drought episodes are the best candidates for starting the dieback. Here I show that *I. acuminatus* may take benefit from the increase of temperature by shifting from one to two generations per year and by escaping winter mortality through overwintering outside of the bark. Although an experimental support is needed, these two adaptations have likely prompted the outbreak in the south-eastern Alps and increased frequency and severity of *I. acuminatus* outbreaks elsewhere in the Alps.

Multiple tree infestations (spots) are typical in bark beetles adopting the cooperative attack strategy. As a result of beetle dispersal, the pattern of spot distribution could be highly dynamic. In *I. acuminatus*, dispersal and attack behaviour change with the generation and this seasonally dependent attack behaviour (spot proliferation in spring and spot growth in summer) is not reported in other European bark beetles. This is typical, however, in the southern pine beetle (SPB, *Dendroctonus frontalis* Zimmermann). As spring dispersal flight is the key to the colonization of new spot and thus to the potential damage, this phase becomes very important to understand how beetles explore distant stands and how they can find suitable trees and form new aggregations. Further studies are needed to clarify the mechanism underlying host searching during this phase. Understanding landscape-scale spatial and spatial-temporal processes of bark beetle infestations is also important when predicting bark beetle activity and behaviour, in particular if control strategies must be applied to limit damage in high value areas, as the valley of Boite.

The sanitation felling carried out in autumn 2007, following the cut and remove method usually applied against many bark beetles species, was not successful despite all the buffer strips were respected and cut. The particular overwintering behaviour of *I. acuminatus*, with adults leaving the trees in late summer, was unknown and it happened that the sanitation felling concerned only a minor part of the beetle population. This specific behaviour adds difficulties in decision making of forest policy managers. The absence of any kind of symptom other than change in colour of the crowns, evident late in the season, makes it impossible to identify the infested trees before beetles swarm and to apply the cutting in the proper time, either in spring or in summer. Research should be addressed to identify alternative control methods, possibly mass trapping if quality of the lures is improved, although the rapid appearance and evolution of bark beetle infestations and the unpredictability of new spot location contribute to make control very difficult.

Moreover, research on control methods should consider the importance of the natural enemies on the population level of *I. acuminatus.* Parasitoids and predators are known to be factors that may limit the spatial spread of an insect outbreak. Our preliminary results demonstrate that parasitoids showed an immediate direct response to the fluctuations of the beetle population, while predators had a delayed and inverse response. Both of them play an important role in determining spot size and extinction, especially at the end of the outbreak. As a consequence of the high pressure that natural enemies exert on the first generation of the beetle, the number of adults swarming in summer and involved in the growth of the size of the spots, is reduced and spot dynamics is deeply affected.

Overall, this study has shown that a multiple approach is required to understand the nature of the outbreak and the population dynamics of *Ips acuminatus*. The results can be generalized to the many stands of *Pinus sylvestris* in the Alps, where the beetle is an important component of the dieback, and to the other species of bark beetles showing a spot dynamics in their attack patterns. Nevertheless, many hypotheses are left open and need further work to be thoroughly addressed.

Acknowledgements

It is a pleasure to thank all those people that have contributed to the work described in this thesis.

First and foremost I want to thank my supervisors, Massimo Faccoli and Andrea Battisti. I really appreciate all their contributions of time and ideas to make my Ph.D. experience productive and stimulating. I owe my deepest gratitude to Martin Schroeder, co-author of some manuscripts, who has given interesting feedback and valuable suggestions and contributions. I am grateful to Marc Kenis, who confirmed the identification of some species of parasitoids and showed me the way to distinguish ‘easily’ species of the same genus. A special thank to Bo Långström, who provided me the fundamental book of Alf Bakke and some valuable articles on parasitoids.

I would like to express my thanks to Valerio Finozzi and Edi d’Ambros (Regional Forest Service) for their precious assistance in field work and data collection. I am grateful to Attilio, a never failing guide for finding a spot in the deepest forest, and Gino. I also sincerely thank Tommaso Anfodillo, Fausto Fontanella and Roberto Menardi (Centro Studi per l’Ambiente Alpino ‘Lucio Susmel’- University of Padova) for providing meteorological data and making field work easier. Thanks to Paolo Paolucci, who transformed a simple crumb hoover in an useful *Ips*-parasitoid-predator-hoover, simplifying consistently insect collection from cages. Moreover, he helped me in taking a lot of photos of parasitoids.

I want to express my gratitude to,Edoardo Petrucco Toffolo, Daniel Zovi and Lorenzo Marini: I really appreciate all the help they gave me in these years. I thank all the entomology staff, professors, technicians and fellows, for the support during the course of my graduate work and I am grateful for time spent with roommates and friends. I thank Caterina T., Elisabetta M., Giovanna, Michele, Nedo and Valentina for their advice, encouragement and patience.

Finally, I would like to thank Marco Guido wholeheartedly, brilliant author of the funny drawings at the beginning of each chapter.