UNIVERSIDAD AUSTRAL DE CHILE
FACULTAD DE CIENCIAS AGRARIAS
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DIVERSIDAD Y CONTROL BIOLÓGICO DE INSECTOS: UN ENFOQUE ECOLÓGICO APLICADO AL CASO DE *Beauveria bassiana* (BALS.) VUILLEMIN, UTILIZADO CONTRA *Dalaca pallens* BLANCHARD (LEPIDOPTERA: HEPIALIDAE).

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Por

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RESUMEN

Cada año, alrededor del 10% de la superficie de praderas del sur de Chile (cerca de 300.000 ha) son asperjadas con insecticidas para controlar la cuncunilla negra de las praderas *Dalaca pallens* (Bl.). Varios de estos insecticidas afectan a más de una especie, acelerando un proceso de pérdida de diversidad iniciado en el siglo XIX cuando los bosques de esta zona del país comenzaron a ser reemplazados por praderas. El control biológico de esta plaga usando esporas del hongo *Beauveria bassiana* (Bals.) Vuill. aislamiento QU-B931 es eficaz, pero se desconoce los efectos que puede tener en el resto de los artrópodos presentes en las praderas. Esta investigación comenzó evaluando la diversidad genética de este hongo mediante la secuenciación de una región intergénica nuclear llamada B locus. Posteriormente, comparó los efectos del insecticida lambda-cyaholotrina y de las esporas del aislamiento QU-B931 en la artropofauna de las praderas, tanto en invierno como en primavera. Esta comparación se realizó en tres diferentes niveles: especies en forma individual (carábidos y arácnidos); gremios (depredadores, herbívoros y descomponedores) y comunidad. Los principales resultados indicaron que una muestra de 97 aislamientos de *B. bassiana*, colectados a lo largo del país, fueron separados en 20 grupos genéticos, algunos de los cuales están restringidos a ciertas regiones del país (por ejemplo Isla de Pascua), mientras que otros están distribuidos a lo largo de Chile. La técnica utilizada permitió discriminar molecularmente el aislamiento de interés del resto de la colección. Por otro lado, la aplicación de lambda-cyhalotrina afectó a cuatro depredadores (dos carábidos y dos familias de arañas), afectó al gremio de los depredadores y disminuyó la diversidad, riqueza de especies y equitabilidad de la comunidad de artrópodos presente en las praderas. Sin embargo, no afectó a dos gremios (herbívoros y descomponedores). En cambio, el control biológico utilizando *B. bassiana* no produjo ningún efecto negativo en la artropofauna. Se concluyó que el control biológico de *D. pallens* es una alternativa con impactos ambientales mucho menores que la actual técnica de control.

ABSTRACT

About 10% of Southern Chile pastures (ca. 300.000 ha) are sprayed with insecticides every year, which are targeted on a native hepialid cutworm, *Dalaca pallens* (Bl.). Some of these insecticides are not species-specific, increasing the biodiversity declining in process since the 19th century, when forests begun to be replaced by pastures. The *D. pallens* biological control using *Beauveria bassiana* (Bals.) Vuill. strain QU-B931 has shown to be as efficient as the current chemical control, but potential non target effects are unknown. The genetic diversity of *B. bassiana* was assessed by sequencing a intergenic nuclear region called B loc. Once the selected strain was characterized, the non target effects of lambda-cyhalothrin and *B. bassiana* spores on the arthropofauna were evaluated in winter and spring. The side effects were evaluated at three different levels: single taxa (carabids and arachnids); guilds (predators, herbivores and decomposers) and arthropod community. A sample of 97 strains collected along the country was separated into 20 genetic groups, some of which are restricted to some regions (for example Eastern Island), while others are distributed across the country. This molecular technique allowed to discriminate the selected strain from the rest of the sample. In the other hand, the insecticide lambda-cyhalothrin affected four predators (two carabid beetles and two spiders), decreased the predator guild and decreased the diversity, species richness and evennes of the whole community. However, it did not affect the decomposer and herbivore guilds. The biological control based on B. bassiana did not affected any species, guild or the community and arises as an alternative with lower environmental effects than the current control.

INTRODUCCIÓN.

El control biológico de plagas enfrenta una nueva realidad a 120 años de su inicio.

El control biológico (CB) de plagas, definido como "la acción de parásitos, depredadores y patógenos para mantener la densidad de otro organismo a un nivel menor de la que tendría en ausencia de esos enemigos naturales" (DeBach, 1964), data desde tiempos antiguos, ya que diversos pueblos han usado insectos para controlar plagas desde varios cientos de años. Sin embargo, el punto de partida del CB como disciplina moderna se remonta apenas a fines del siglo XIX (1888), con la importación y liberación desde Australia a California de un insecto (*Rodolia cardinalis* Mulsant) para controlar una plaga (*Icerya purchasi* Maskell) que amenazaba seriamente la industria de cítricos en ese estado. Esta introducción tuvo un éxito resonante, atrayendo la atención de muchos e impulsando la nueva técnica.

DeBach (1974) ha estimado que el 99% de las potenciales plagas de los cultivos están controladas por sus enemigos naturales. En cien años, se han realizado alrededor de 5000 introducciones de casi 2000 artrópodos en 200 países e islas utilizando el enfoque del CB clásico, las que raramente han producido efectos negativos (van Lenteren et al., 2006). En adición, el CB inundativo ha usado durante 90 años unas 150 especies de enemigos naturales para controlar alrededor de 100 plagas, sin producir efectos negativos notorios o irreversibles.

En base a lo anterior, el CB ha sido visto como una de las técnicas de control de plagas más seguras desde el punto de vista ambiental y la que supone menos riesgos para otras especies (DeBach, 1974; Batra, 1982; Moon, 1982; Hokkanen y Pimentel, 1989; van Lenteren et al., 2005). Esta característica ha sido una de los razones, junto a la excelente relación costo/beneficio, que explican la expansión, el interés permanente de los usuarios y la imagen amigable del CB entre el resto de la sociedad no involucrada directamente con las actividades agrícolas.

Sin embargo, a pesar de esta percepción de seguridad, la inquietud acerca de los efectos indeseados del CB surgió casi simultáneamente con su aparición. Howarth (1991) ejemplifica cuán antigua es esta preocupación al transcribir una carta fechada en 1899, entre David Sharp y L. O. Howard, acerca del programa para importar enemigos naturales en Hawaii:

"…Debería asegurarse un registro permanente de lo que el señor Koebele ha realizado en materias que pueden afectar la fauna y estaríamos muy agradecidos si Ud. redactara una orden sobre estos puntos tan pronto como pueda. El señor Koebele está haciendo realmente un enorme experimento biológico y los detalles deberían ser completamente registrados, ya que es sabido que deberá pasar mucho tiempo antes que todos los resultados puedan ser estimados exactamente." (D. Sharp, 1899).

Esta posición crítica, basada inicialmente en la prudencia, se vio reforzada cuando en la primera mitad del s. XX el CB tuvo algunos rotundos fracasos, especialmente cuando fueron utilizados vertebrados (van Lenteren et al., 2005). La corrección oportuna de estos errores no eliminó por completo las aprensiones y la controversia en cuanto al verdadero alcance de los potenciales efectos negativos del CB ha continuado durante el último medio siglo, con autores que defienden el CB como una técnica de control de plagas eficiente, de bajo costo y segura ambientalmente (DeBach, 1974; Batra, 1982; Moon, 1982; Hokkanen y Pimentel, 1989; DeBach y Rosen, 1991; van Lenteren et al., 2005), mientras que otros autores expresan dudas acerca de la seguridad del CB, documentando varios ejemplos de efectos no deseados producto de la aplicación de esta técnica (Pimentel et al., 1989; Tiedje et al., 1989; Simberloff, 1992; Lockwood, 1993; Lockwood, 1996; Simberloff y Stiling, 1996).

Debido a la importancia del tema, la ausencia de evidencia de impactos ambientales negativos no debería considerarse como prueba para rechazar la existencia de esos impactos (Howarth, 1991), más aún cuando información que había sido recopilada inicialmente en estudios no relacionados directamente con el tema ha servido para realizar estudios más sistemáticos (Hadfield y Mountain, 1981; Murray et al., 1988). Como resultado, la

documentación de impactos negativos significativos ha aumentando y con ello la percepción del problema (Ehler, 1990; Harris, 1988; Howarth, 1983; Pimentel et al., 1984; Murdoch et al., 1985; Roberts, 1986; Hadfield, 1986; Hintz et al., 2001).

En forma paralela a la mayor evidencia empírica de efectos no deseados, la concepción de seguridad ambiental ha evolucionado en el tiempo (Sheppard et al., 2000) y los valores predominantes en la sociedad de fines del s. XIX y principios del s. XX no son los mismos que en el presente. Estos nuevos valores se manifiestan, entre otros aspectos, por los siguientes:

a) Aumento de la preocupación por la biodiversidad: se valora en forma diferente a organismos que anteriormente no eran considerados directamente beneficiosos (Simberloff, 1992; Lockwood, 1993). Si existen poblaciones residentes (nativas) del agente de control biológico (ACB) que se desea utilizar, la posible pérdida o disminución en la frecuencia de ciertos genes también supone un efecto indeseable (Hintz et al., 2001).

b) Mayor desarrollo teórico y experimental de las diferentes ramas de la ecología: el control biológico de plagas, en su concepto moderno, surgió y maduró en una época en la que el cuerpo teórico de la ecología de poblaciones y de comunidades estaba en sus albores o simplemente no existía. Pero en los últimos 50 años, estas disciplinas han avanzado velozmente tanto en el aspecto teórico como en el aspecto metodológico y existe un creciente consenso en el sentido que el CB seguirá siendo una tecnología competitiva sólo en la medida que incorpore y se apoye en las disciplinas nombradas anteriormente (Thomas et al., 2004; Naeem y Wright, 2003).

Formas de enfrentar el problema.

El paradigma inicial para evaluar los efectos del CB fue considerar que la posibilidad de impactar especies no plaga dependía casi exclusivamente de la especificidad del agente. La consecuencia directa de este paradigma es que agentes altamente específicos supondrían una amenaza mínima para especies distintas a la plaga e, inversamente, agentes con un rango amplio de hospederos o presas supondrían una amenaza mayor (Goettel, 1995). La

preeminencia de esta idea ha sido extrema en el caso del CB de malezas, donde los potenciales agentes candidatos deben sortear severas pruebas de especificidad antes de ser aprobados y se refleja en el esquema propuesto por Wapshere (1974), conocido como “método filogenéticamente centrífugo”.

Esta idea ha sido desafiada por un número creciente de trabajos que entregan evidencia teórica, observacional y experimental acerca de los efectos que pueden producir los agentes de CB, inclusive aquellos altamente específicos (Lynch et al., 2002). Producto de lo anterior, la determinación del grado de especificidad del ACB aún es necesaria pero no suficiente para evaluar adecuadamente la posibilidad de efectos no deseados del CB (Kuhlmann et al., 2005; van Lenteren et al., 2005). Paulatinamente se está reconociendo que un ACB puede establecer diferentes y numerosos tipos de interacciones no sólo con la especie que se desea controlar, si no que, más frecuentemente de lo pensado, con el resto de las especies que constituyen la comunidad donde el ACB va a ser utilizado.

A partir de este reconocimiento, se hace patente que evaluar los potenciales efectos del CB se dificulta debido a la complejidad inherente a las comunidades donde se pretende implementarlo. El número de especies que coexisten en los agroecosistemas, incluidas las praderas permanentes del sur de Chile, hace presumir la existencia de múltiples interacciones entre ellas, algunas más evidentes que otras. Dado que el CB es una intervención deliberada para obtener cierto resultado en la composición y en la dinámica de determinados ensambles, puede considerarse como una aplicación particular de la ecología de poblaciones, ecología de comunidades y del paisaje. Por lo tanto, puede nutrirse del cuerpo teórico desarrollado por estas disciplinas (Louda y Arnett, 2000).

El control biológico como un servicio ecológico.

Reiterando que el CB es una tecnología que se desarrolló antes que las disciplinas que le entregan sustento teórico, surgen distintas formas de enlazar o establecer el vínculo entre ellos. El CB manipula deliberadamente cierta(s) especie(s) con el fin de influir en la dinámica poblacional de otra especie. Por otro lado, estas adiciones/sustracciones de

especies y/o los cambios en sus poblaciones crean la posibilidad de influir en la dinámica de especies fuera del objetivo del CB.

El punto central de la evaluación del CB consiste en aumentar la capacidad de predecir los potenciales cambios en una comunidad producto de su uso, antes de llevarlo a cabo. Swift et al., (2004) definen “funciones ecosistémicas” como el conjunto agregado mínimo de procesos (incluyendo aquellos de tipo bioquímico, biofísico y biológico) que aseguran la productividad biológica, la integridad organizacional y la perpetuación del ecosistema. Las funciones ecosistémicas son numerosas e incluyen el movimiento de la energía a través de las cadenas tróficas, la transferencia y reciclaje de nutrientes, descontaminación del aire y el agua, entre muchas otras. La regulación de los herbívoros, incluyendo los insectos, es la función alrededor de la cual gira esta investigación y cómo podría verse afectada por los potenciales cambios producidos por las diferentes técnicas de control usadas contra un insecto herbívoro. A partir del reconocimiento que la regulación de los herbívoros es una más de las funciones necesarias para el funcionamiento de los ecosistemas, si adoptamos una posición antropocéntrica esta función ecosistémica puede ser vista como un “servicio ecológico”, ya que el hombre depende de la mantención del ecosistema para su propio bienestar. De esta forma, el control natural de insectos sería uno de los procesos necesarios para el correcto funcionamiento de un ecosistema particular, en este caso de tipo antropogénico (las praderas del sur de Chile).

El funcionamiento de los procesos ecológicos ha sido vinculado a numerosas propiedades, incluyendo la conectancia, el largo de las cadenas tróficas, la presencia de especies clave, etc. Para todos los factores mencionados existe evidencia teórica y experimental que los sustentan, dependiendo del sistema que se trate (marino, lacustre, intermareal, terrestre), de la escala temporal, de la escala espacial y del tipo de comunidad biótica, que explica por qué algunas predicciones o hipótesis son válidas en algunos casos y no en otros.

Por otro lado, según la terminología usada por Schlapfer y Schmid (1999), los “efectos en el ecosistema” son aquellos efectos en las propiedades o procesos medidos en los componentes bióticos o abióticos del mismo, siempre que sean observables en la escala de

niveles tróficos completos o grandes grupos taxonómicos que representen una gran proporción de la biomasa total dentro de su nivel trófico.

En años recientes ha surgido con fuerza el debate acerca de un posible vínculo entre la diversidad de un sistema y el funcionamiento del mismo. Mientras algunos investigadores manifiestan su desacuerdo frente a vincular la diversidad *per se* con el funcionamiento y la estabilidad de los ecosistemas (Bengtsson, 1998), a medida que la diversidad continúa disminuyendo, más esfuerzos se dedican a evaluar su importancia para el funcionamiento, la estabilidad de los ecosistemas y la provisión de "servicios ecológicos" (Schwartz et al., 2000), entre ellos el control biológico de plagas. Varios autores vinculan la provisión de servicios ecológicos con la diversidad y extienden esta relación al caso particular de la diversidad de enemigos naturales y el control biológico de plagas (Risch et al., 1983; Wilby y Thomas, 2002b).

Fruto de lo anterior, se acumula evidencia que a medida que un sistema agrícola se intensifica y utiliza más aportes externos, tiende a perder diversidad, a desestabilizarse y como consecuencia aumenta la frecuencia y magnitud de los brotes de plagas (Altieri, 1991; Swift et al., 1996). Sin embargo, se conoce poco sobre los mecanismos que explican la desestabilización o cómo los impactos de la actividad agrícola en la biodiversidad influyen en el control natural de plagas. En general, los patrones de emergencia de plagas permanecen pobremente explicados (Wilby y Thomas, 2002a) y esta falta de explicaciones mecanísticas ha contribuido a debilitar los argumentos a favor de la relación diversidad-funcionamiento de los ecosistemas.

Sin embargo, incluso aquellos investigadores más críticos reconocen que una alta diversidad es deseable como una fuente de especies que realizan funciones o dan servicios a medida que cambian las necesidades humanas o las condiciones ambientales (Bengtsson, 1998), e incluso algunos consideran este hecho como una función más de la diversidad (Folke et al., 1996; Walter, 1991; Wellnitz y Poff, 2001).

Relacion diversidad/funcionamiento como punto de partida.

Las praderas del sur de Chile son el resultado del proceso de deforestación realizado por el hombre con el fin de disponer de tierras para el pastoreo. A partir de un paisaje compuesto por bosques de diferentes especies nativas, hoy en día la vegetación dominante está compuesta por gramíneas forrajeras y las especies arbóreas que dominaban el paisaje antaño están relegadas a fragmentos de bosque, alrededor en los cursos de agua que corren por los predios o árboles aislados en medio de las praderas (Durán, 1976)..

Este proceso inducido por el hombre ha significado un cambio fundamental en la composición de la comunidad de artrópodos presentes en ellas. Sin embargo, estos cambios han sido poco estudiados y no se conoce con certidumbre cómo cambió la diversidad de insectos ni menos en qué punto se encuentra este agroecosistema en relación a la proporción de diversidad necesaria para su adecuado funcionamiento, asumiendo que efectivamente existe esa relación.

Pese a ser un sistema simple, las praderas albergan una gran cantidad de especies de artrópodos, en diferentes niveles tróficos. Desde el punto de la producción agrícola, los insectos herbívoros de mayor importancia son la cuncunilla negra de las empastadas *Dalaca pallens* Blanchard (Lepidoptera: Hepialidae), el gorgojo argentino de las ballicas *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) y gusanos blancos tales como *Phytoloema herrmanni* Germain, *Hylamorpha elegans* (Burmeister), *Brachysternus prasinus* Guerin (Coleoptera: Scarabaeidae), larvas de gusanos alambre *Medonia deromecoides* Schwartz (Coleoptera: Elateridae) y larvas de dípteros *Chiromyza paulseni* (Phil.) (Diptera: Stratiomyiidae). Otras especies son conocidas por sus hábitos depredadores y se les considera como especies benéficas, al igual que numerosos parasitoides. Por otro lado, otros herbívoros raramente han superado el umbral de daño económico y por lo tanto han recibido escasa atención, al igual que muchas especies que por su hábito trófico corresponderían a descomponedores, fungívoros o consumidores de polen.

Se ha propuesto diversos enfoques para evaluar los efectos del control de plagas en el funcionamiento de los ecosistemas, incluyendo enfoques tales como el estudio de los gremios que dependen de la especie plaga (Louda y Arnett, 2000); realizar el análisis basado en los grupos funcionales presentes en el agroecosistema (Bengtsson 1998) o concentrarse en los llamados módulos comunitarios (Hochberg et al., 1996; Holt, 1997; Holt y Hochberg, 2001).

El conocimiento actual de las comunidades de artrópodos en las praderas es muy parcial, tanto desde el punto de vista taxonómico como funcional. Estudios de contenido intestinal y de exclusión (Morales, 2000; Espíndola, 2004) han demostrado la relación entre áfidos y sus depredadores carábidos en cereales, pero las relaciones tróficas entre las especies presentes en las praderas son prácticamente desconocidas. Por ejemplo, Prado (1991) en su catálogo de las plagas chilenas no nombra ninguna especie que deprede sobre *D. pallens* y sólo señala la existencia de taquínidos que parasitan a este hepiálido. Otra obra fundamental de la entomología económica (Artigas, 1994) se refiere a la existencia de "depredación por aves, roedores y carábidos sobre las cuncunillas negras", sin entregar más antecedentes sobre el tema.

La ausencia de estos antecedentes limita seriamente la posibilidad de aplicar los enfoques propuestos como alternativa a la medición de la diversidad total. Los módulos comunitarios por definición deben incluir un número pequeño de especies (3-4) que se interrelacionen con tal fuerza que puedan ser analizados en forma aislada del resto de la comunidad (Holt y Hochberg, 2001). Al desconocerse cuáles especies están relacionadas con *D. pallens* y la relativa fuerza de esta potencial interacción, la posibilidad de identificar un módulo que incluya a *D. pallens* se reducen drásticamente. La definición de grupos funcionales en las praderas puede realizarse en base a los hábitos tróficos deducidos a partir de la morfología de las distintas especies, pero los límites exactos de tales grupos también presentar dificultades para ser determinados ya que especies que se presumen depredadores también incluyen material vegetal en sus dietas e incluso sus presas incluyen especies ubicadas en

distintos niveles tróficos, en proporciones que varían de especie en especie (Espíndola, 2004).

Por lo tanto, la aplicación de enfoques más mecanísticos tales como los descritos en el párrafo anterior se ven severamente limitados por el estado actual del conocimiento del funcionamiento de las praderas, produciendo que el enfoque más factible de aplicar sea medir los cambios en la diversidad total, reconociendo las limitaciones que este enfoque posee, resumidas en lo que Bengtsson (1998) describe como ausencia de mecanismos explícitos que expliquen cómo los cambios en la diversidad se reflejan en un mejor o peor funcionamiento de la regulación de los herbívoros.

Descripción de la plaga y su control.

Las cuncunillas negras de las praderas son un complejo formado por los estados larvales de tres especies de hepiálidos: *Dalaca pallens*, *D. chiliensis* (Viette) y *D. variabilis* (Viette). Aunque difíciles de diferenciar en los estados inmaduros, en general la primera de las especies nombradas es la más común y usualmente corresponde a más del 80% de los individuos (Cisternas, comunicación personal). Los adultos de la familia son conocidos en la literatura de habla inglesa como polillas fantasma debido a la coloración blanca de los machos, su vuelo crepuscular y en suspensión. El ciclo de la especie comienza con los huevos, los cuales son depositados en grandes cantidades sobre las praderas, aparentemente sin un patrón definido, durante los meses de enero a marzo. Las larvas que eclosan de ellos construyen galerías verticales en el suelo, en las que la larva permanece escondida durante el día. En las horas de oscuridad, la larva deja la galería y recorre la superficie de la pradera consumiendo hojas, culmos, corona y parte superior de las raíces de las plantas forrajeras. Al acercarse el día, las larvas vuelven a sus galerías gracias a un hilo de seda que secretan y se refugian hasta la noche siguiente. Las larvas se desarrollan desde febrero a noviembre, período en el que consumen una gran cantidad de material vegetal y reducen severamente el rendimiento de la pradera. En casos extremos, el debilitamiento de las plantas incluso puede acarrear la muerte de las mismas. Se cree que los adultos son capaces de volar grandes distancias, entre los meses de enero a marzo.

El control de esta especie se realiza mayoritariamente utilizando insecticidas químicos, los cuales se aplican preferentemente en otoño. Los insecticidas más utilizados corresponden a dos grupos: reguladores de crecimiento y piretroides, entre ellos el insecticida lambda-cyhalotrina.

El agente de control.

El hongo *Beauveria bassiana* (Balsamo) Vuillemin *sensu lato* se ha definido como una especie en base a caracteres morfológicos y alberga aislamientos que se diferencian ampliamente en cuanto a patogenicidad y otros aspectos, al extremo que algunos autores han propuesto la existencia de especies crípticas al interior de ella (Rehner y Buckley, 2005). Este hongo es un saprófito facultativo y posee propiedades patogénicas que han atraído interés hacia la especie desde fines del siglo XIX. El rango de hospederos, considerando la especie como un todo, incluye más de 700 especies de artrópodos (Li, 1988), lo que explica el gran interés que ha atraído por su potencial como controlador biológico de plagas. No obstante lo anterior, la virulencia y especificidad varían considerablemente entre aislamientos (Lecuona et al., 1996), lo cual sugiere una base genética diferente (Berreta et al., 1998).

Este hongo deuteromycete tiene una distribución cosmopolita y es un habitante común del suelo, aunque también puede encontrarse al interior de plantas como endófito, en el filoplano o en sustratos distintos al suelo. Se reproduce en forma asexual mediante dos tipos de esporas (conidias y blastosporas) y permanece en estado haploide durante casi toda su existencia. La reproducción sexual es un fenómeno escasamente observado en la especie (Zenghi et al., 2001), aunque presenta otros mecanismos de intercambio de material genético (parasexualidad).

El proceso de patogénesis comprende varias etapas, cuyo número varía dependiendo del autor, pero que pueden resumirse de la siguiente manera: adhesión de la espora a la cutícula del artrópodo; germinación de la espora; emisión del haustorio; rompimiento de la cutícula por medio de la hifa de penetración; diseminación al interior del hospedero por medio de

blastosporas; liberación de toxinas y muerte del hospedero; producción de micelio; y producción de nuevas esporas.

En Chile se ha recolectado más de 600 aislamientos de *B. bassiana*, algunos de los cuales poseen una alta patogenicidad hacia artrópodos plagas de importancia para nuestro país (France et al., 2000; Gerding et al., 2000). En el caso particular de *D. pallens*, el aislamiento QU-B931 fue obtenido a partir de larvas de esta especie que se encontraban infectadas en una pradera en las cercanías de Osorno, Décima Región. Pruebas de laboratorio y de campo, tanto a pequeña como gran escala, han demostrado que el nivel de control de cuncunilla negra utilizando este hongo es similar al nivel de control alcanzado con el uso del insecticida lambda-cyhalotrina (Cisternas et al., 2003).

Sin embargo, el control utilizando esporas del aislamiento QU-B931 presenta particularidades que lo diferencian de otros tipos de control:

1. El CB mediante este tipo de microorganismos se ha realizado preferentemente utilizando el enfoque inundativo (Bellows et al., 1999), es decir, con la aplicación masiva y concentrada de grandes cantidades de propágulos del microorganismo en un ambiente, sin que necesariamente se establezca ni propague. Esto implica que cada vez que sea necesario se realiza una nueva aplicación.
2. Entre los agentes utilizados en CB, la reproducción sexual es más común en los macroorganismos, mientras que la reproducción asexual predomina en los microorganismos. Lo anterior implica que la diversidad genética de los microorganismos utilizados en CB generalmente sea menor que la diversidad genética de los macroorganismos usados en CB.

La primera de estas singularidades (predominancia del enfoque inundativo) implica que los hongos entomopatógenos generalmente son utilizados en ambientes donde ya estaban presentes, pero en menor cantidad. Por lo tanto, su uso no implica la expansión de su rango geográfico, pero sí involucra la posibilidad de desplazar poblaciones locales conespecíficas,

con la consiguiente pérdida o disminución de algunos genes (Hintz et al., 2001; Teng y Yang, 1993).

La segunda de estas consideraciones (predominancia de la reproducción asexual) implica que la identificación individual de un genotipo seleccionado, en el caso de un hongo entomopatógeno, suele ser más difícil que en otro tipo de organismos utilizados en control biológico. Los aislamientos seleccionados para ser usados como insecticidas microbianos, tal como el aislamiento QU-B931, requieren ser caracterizados a un nivel infra-específico (Jenkins y Grzywacz, 2000), ya que aislamientos diferentes muestran una considerable especialización en cuanto a su hospedero y por ende la identificación a nivel de especie resulta insuficiente (Humber, 1997).

La escasez de caracteres morfológicos confiables en el género *Beauveria* dificulta distinguir entre especies y sobre todo entre aislamientos al interior de la especie. Lo anterior ha impulsado la búsqueda de otros caracteres taxonómicos, en especial de tipo molecular: isoenzimas, RFLP mitocondrial, inmunología, secuencias de rRNA, RFLP, RAPD, microsatélites, secuencias de la región ITS e intrones de la subunidad mayor del rDNA (St Leger et al., 1992; Piatti et al., 2000; Valderrama et al., 2000; Bidochka et al., 1994; Castrillo y Brooks, 1998; Rehner y Buckley, 2005).

Antecedentes sobre los efectos no deseados producidos por el uso de *Beauveria* spp.

A pesar que cerca del 50% de casos de aplicaciones inundativas de microorganismos entomopatógenos han producido algún tipo de efecto no deseado en otras poblaciones, ellas son consideradas suficientemente seguras debido a su relativa falta de persistencia y el consecuente carácter temporal de sus impactos negativos (Lynch y Thomas, 2000).

El enfoque tradicional para evaluar este tipo de efectos ha incluido realizar pruebas de patogenicidad del aislamiento seleccionado hacia especies de interés particular (rango de hospederos potencial o máximo) y posteriormente evaluar el efecto a nivel de campo en esas especies (rango de hospederos realizado) [Amano y Haseeb, 2001]. Siguiendo este esquema de evaluación, se ha reportado que algunos aislamientos de *Beauveria* spp. han

causado efectos negativos en especies no plaga, mayormente especies consideradas benéficas, en especial depredadores y parasitoides (Traugott et al., 2000; Danfa y van der Valk, 1999; Jayanthi y Padmavathamma, 1996; Brinkman y Fuller, 1999; Steenberg et al., 1995; Wang et al., 2001; Wang et al., 2004).

El esquema descrito anteriormente presenta deficiencias que impiden minimizar las posibilidades de producir efectos indeseables en especies distintas a la plaga:

1. La selección de las especies no plaga incluidas en la evaluación presenta un claro sesgo hacia especies del tercer nivel trófico (depredadores y parasitoides). Desde un punto de vista antropocéntrico, estas especies son altamente valoradas debido a su contribución a la regulación de los herbívoros. Sin embargo, no se valora adecuadamente a especies que también contribuyen al funcionamiento del control biológico, actuando como presas alternativas en períodos en los que la plaga escasea (Hardin et al., 1995). Especies vinculadas a otros procesos ecosistémicos, tales como el reciclaje de nutrientes o el movimiento de la materia y la energía a través de las cadenas tróficas, tampoco han estado suficientemente representadas en este tipo de estudios.
2. Prácticamente la totalidad de estos estudios han sido conducidos considerando las especies en forma individual y no se ha asumido suficientemente el impacto sobre grupos de especies relacionadas, es decir, no se han incluido el rol de grupos funcionales en lugar del impacto de especies aisladas (Simberloff y Dayan, 1991).
3. Si son pocos los estudios que han evaluado los efectos no deseados a mediana escala (grupos funcionales), los estudios que han abordado este tópico desde una perspectiva mayor son aún más escasos. Antecedentes del impacto del control biológico usando *B. bassiana* u otro hongo entomopatógeno en las propiedades (diversidad, riqueza de especies, equitabilidad) de la comunidad de artrópodos son prácticamente inexistentes.

4. Numerosos estudios se concentran en interacciones directas, especialmente tróficas, y carecen de antecedentes sobre potenciales efectos indirectos (Simberloff y Stirling, 1996; Lockwood, 1996). Las interacciones tróficas están lejos de ser la única manera de cómo una especie puede influir en la dinámica poblacional de otra. Las interacciones indirectas pueden ser tan importantes en magnitud como las interacciones directas en la composición de un ensamble de especies y en la dinámica de sus miembros (Pearson y Callaway 2003), con efectos observables incluso en escalas de tiempo cortas (Menge, 1997). Los mecanismos propuestos son variados e incluyen los subsidios (un ACB especialista puede ser consumido por organismos generalistas residentes; Nouhuys y Hanski, 2000), las respuestas compensatorias y el reemplazo ecológico (Pearson y Callaway, 2003).
5. En general, pocos estudios estudian simultáneamente los efectos no deseados, la persistencia y la dispersión de las esporas. Este tipo de información es necesaria para evaluar los potenciales riesgos en una adecuada escala temporal y espacial.
6. Contar con herramientas para discriminar los aislamientos seleccionados de otros aislamientos, en especial aquellos ya presentes en el lugar de aplicación, contribuiría a una mejor evaluación de este tipo de efectos (Goettel, 1995) y a estudiar potenciales cambios en la diversidad genética de la población residente del ACB (Hintz et al, 2001).

Cumplir con cada uno de los puntos mencionados anteriormente significaría un avance para superar lo que diversos autores definen como carencia de protocolos confiables para la evaluación de los enemigos naturales y baja capacidad de predecir el desempeño de un ACB y sus riesgos (Simberloff y Stirling, 1996; Lynch y Thomas, 2000).

En conclusión, una sociedad más receptiva a las cuestiones ambientales y menos tolerante a la producción agrícola industrial indiscriminada crea demandas de información mucho más amplias que en el pasado. Como consecuencia de lo anterior, actualmente la evaluación del CB va más allá de la determinación del rango de hospederos o de la especificidad del

agente y es muy probable que esta presión social exija realizar completos análisis de riesgo-beneficio para todo organismo que sea postulado como agente de control y que su uso sea autorizado sólo cuando los beneficios sobrepasen con creces los riesgos para las especies nativas y benéficas. Esta situación ya se ha hecho realidad, tal como ocurre en algunos países como Nueva Zelanda (Sheppard et al., 2000) o acciones concretas como la iniciativa ERBIC (Environmental Risks of Biological Control Introductions into Europe) en la UE para evaluar los efectos no deseados del CB y crear metodologías que permitan anticiparlos (Lynch y Thomas, 2000).

La hipótesis central plantea que la nueva técnica de control producirá menores efectos no deseados que la aplicación de los insecticidas actualmente en uso. En consecuencia, esta investigación fue desarrollada para intentar responder las siguientes preguntas:

¿Cuánta variación genética existe al interior de las poblaciones chilenas de *B. bassiana*? ¿Cómo se distribuye esta variación? ¿Es posible identificar factor(es) que expliquen esta potencial estructura genética?

¿Cuáles son los efectos de la aplicación masiva de esporas de *B. bassiana* en la artropofauna presente en las praderas? ¿Cómo se comparan estos potenciales efectos con aquellos causados por la(s) actual(es) técnica(s) de control? ¿Por cuánto tiempo pueden persistir las esporas en el suelo y en las plantas?

1.- CAPÍTULO PRIMERO: DIVERSIDAD GENÉTICA E IDENTIFICACIÓN DEL AGENTE DE CONTROL BIOLÓGICO.

Este artículo puede ser consultado bajo el título:

Devotto L., S.A. Rehner, M. Méndez and A. France (in prep.). Genetic diversity of *Beauveria bassiana* (Balsamo) Vuillemin in Chile revealed by a nuclear gene segment sequencing.

GENETIC DIVERSITY OF *Beauveria bassiana* (BALSAMO) VUILLEMIN IN CHILE REVEALED BY A NUCLEAR GENE SEGMENT SEQUENCING.

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Summary.

The genetic structure of seven Chilean putative populations of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin was investigated by sequencing a ca. 1400 bp nuclear gene fragment named B locus. Ninety seven *B. bassiana sensu lato* isolates were included, which have been previously collected accross the country, most of them from soil using the *Galleria mellonella* baiting method. Phylogenetic and demographic approaches were adopted in a sequential fashion to analyze the resulting data set. The 97 isolates were collapsed to 20 haplotypes. Neighbour-joining and maximum parsimony trees revealed 9 well supported clades. Only one clade included isolates collected in the same locality and they corresponded to Eastern Island isolates. The remaining clades gathered isolates from very different geographical origin, including isolates thousands of kilometers apart. Seven populations were defined *a priori* based on ecological and climatic conditions. The AMOVA analysis revealed a significant but limited support for the proposed structure. One isolate, coded QU-B931, is a good potential candidate for biological pest control. This isolate was distinguishable from the rest of the

sample, showing the potential use of this nuclear fragment for strain fingerprinting. A eleven sub-sample, representing the major clades, was additionally analized by sequencing the elongation factor 1 alpha (EF1-α) gene. The resulting EF1-α topology mostly agreed with the B locus topology and allowed to analyze the genetic diversity of the fungus in Chile under current phylogenetic hypotheses for the *B. bassiana* complex, which suggest the existence of cryptic species into what is morphologically defined as *B. bassiana*.

Keywords: entomopathogenic fungus; biological control; population structure; elongation factor 1 alpha.

Introduction.

The haploid filamentous fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a common soil inhabiting organism, consuming organic matter (saprophytic stage) and/or infecting arthropods (parasitic stage). Its pathogenic properties have been long recognized and have attracted much effort to use this fungus as a biological control agent against many agricultural, medical and veterinary important pests. Several biological control programmes based on this fungus have successed in recent years, but in other cases more erratic results have been obtained. Some of these failures could have been explained and overcome if a better understanding of life traits, ecological interactions and phylogenetic constraints were available.

The efforts directed to understand the above mentioned issues are limited by the lack of enough reliable phenotypic characters to discriminate among species into the genus and among strains into the species. The genus *Beauveria* harbours several species defined mostly by the conidia morphology, while strain identity at infra-species level is even more unclear if morphology is the only criteria.

The species and strain identity is an integral part of any environmental risk and efficacy assessment structure. In consequence, biochemical and molecular tools have been used to fingerprint strains of particular interest. The mitochondrial DNA has been the preferred target of infra-species studies because of its faster evolution rate, but recently the nuclear DNA has arose as another valid alternative. Nuclear genes are suitable for intraspecific studies as they serve as nonlinked genetic markers, converse to mitochondrial genes which

act as single locus (France et al., 1999). Therefore, both kinds of genes differ in their rates of evolution and modes of inheritance.

The genetic structure arises from the interaction of several factors, some of which (mutations, genetic drift and, in some cases, selection) increase variation, while other like gene flow tend to homogenisation. This balance also can be influenced by species life traits, for example the fungus mating system. The species belonging to the *Beauveria* genus reproduce by two asexual spores: the yeast-like spores are produced into the host during the pathogenesis process, while conidia are produced over the host surface after the host death. Sexual reproduction is a very rare event and when it has been observed, the resulting fruit bodies have shown similarity with the genus *Cordyceps*, but some genetic material is exchanged by other ways such as the parasexual cylce, in which two compatible hyphae fuse temporally. In brief, the limited genetic exchange and dispersal capability of *B. bassiana sensu lato* allow supposing a strongly structured population.

The growing importance of integrated pest management (IPM) and biological pest control have triggered efforts to use native entomopathogenic organisms in Chile. In the late 1990s, the Entomopathogenic Organisms Collection arose from a nation-wide prospecting and collecting effort, including nematodes and fungi such as *Metarhizium*, *Verticillium*, *Cordyceps* and *Beauveria* spp. About 300 *B. bassiana* isolates were collected from agricultural and natural systems, different regions and hosts. Consequently, the potential of this entomopathogenic fungus is being evaluated and so far, up to 30 insect hosts have been identified, most of them important pests of main Chilean crops, confirming the wide host range of the species and the extreme variability between genotypes, at least what pathogenicity concerns. Nevertheless, pathogenic properties of genotypes are only partially characterized and other relevant ecological and genetic traits remain unknown.

The Chilean *Beauveria bassiana* isolates were found in very contrasting environments and ecological conditions, from the desertic areas through Mediterranean and temperate zones until Patagonia, including Eastern Island. Such wide distribution of a clonal organism poses a interesting fact from both basic and applied ecology. In consequence, this study was conducted to determine genetic diversity of *B. bassiana* accross its observed range in Chile

and put it in a phylogeographical perspective, relating any potential pattern with geographical origin and other relevant traits of the isolates. In addition, the second aim was to assess the potential use of nuclear gene sequencing for strain fingerprinting.

Materials and methods.

We adopted a three-step sequential approach to examine the genetic variation of Chilean *B. bassiana* populations. The rationale and background of this approach are given in detail by Bernatchez (2001) and Althoff and Pellmyr (2002). The first step was to examine the potential patterns of relatedness among haplotypes by standard phylogenetic analyses (phylogenetic trees and haplotype network). Then, we incorporated analyses of demographic history such as mismatch distributions and surveys of haplotype and nucleotide diversity. Finally, we performed population genetic analyses to deal with recent population structure: distance pairwise comparisons and AMOVA seem particularly suitable to provide insights into genetic structure at this level.

Sample collection.

Over three hundred *B. bassiana* accessions are hold in the Entomopathogenic Organisms Collection at Instituto de Investigaciones Agropecuarias (INIA), Ministry of Agriculture, Chillán, Chile. This collection has been built since 1997, collecting soil samples across Chile. The preferred isolation method was *Galleria mellonella* baiting, described by Alves et al., (1998). Some other accessions have been obtained directly from parasitized individuals. Strains were preserved under –196 ºC.

Isolate selection and populations.

A total of 97 isolates, morphologically identified as *Beauveria bassiana* (Balsamo) Vuillemin, were selected to be included in the study. They were selected with the aim to represent contrasting ecological conditions, natural and anthropogenic habitats, insect host and pathogenicity, when they were available. Based on geographic and ecological characteristics, seven hypothetical populations were defined. Names, number of isolates and climatic data for each population are shown in Table 1. Monosporic cultures were obtained through dilution plating method, inoculating Petri dishes with diluted PDY medium (one-quarter strength). A diluted suspension of spores was spread on a thin layer of

medium and single spores were identified under a microscope (40X). A block of medium (2x2 mm) was excised from the dish and transferred to new dishes containing undiluted PDY media (20 g agar, 10 g dextrose, 2.5 g peptone, 2.5 g yeast extract L^{-1}). Dishes were kept to 22º C until complete sporulation was observed. Relevant information of each strain is listed in Table 2.

Molecular methods.

Molecular data was generated by sequencing a region of ~1500 base pairs (bp), named B fragment. A subset (11 strains), representative of major clades revealed by B fragment analysis, was additionally analyzed by sequencing ~1600 (bp) of elongation factor 1 alpha (EF1-α) gene as described in Rehner and Buckley (2005).

DNA extraction.

Erlenmeyer flasks containing 50 mL of PDY broth (as described above, but agar) were inoculated with agar pieces from Petri dishes and cultured 3-5 days in orbital shakers (25º C, 150 rpm). Then, broth and tissues were centrifuged (15 min, 8000 x g). Supernatant was discarded and pellet re-suspended in distilled water (25 mL) and centrifuged again (15 min, 8000 x g). Packed tissues were cooled to –80 ºC for 30 min and lyophilized overnight.

Total genomic DNA was extracted as follow: the lyophilized tissue pieces (about 0.2 mL) were broken into smaller pieces and shaken for 12 s in a FastPrep bead beater (Savant Instruments Inc., Farmingdale, NY). From this step, the procedures followed Rehner and Buckley (2005), with slight changes.

Once the mycelium was ground, 700 μL of extraction buffer (described in Rehner and Buckley, 2005) were added. The tubes were shaken in the bead beater (3 s at speed setting of 4) and incubated at 55 ºC for 10 min in a heat block. After incubation, 600 μL CIA (24:1 chloroform:isoamyl alcohol) were added to each tube and mixed by hand until an emulsion was formed, centrifuged (20000 x g, 5 min) to separate the cellular debris. The upper layer (ca. 700 μL) was transferred to a new tube and 700 μL of 6 M guanidinium thiocyanate were added, mixed and 10-15 μL of glass powder suspension was added. Tubes were incubated at room temperature for 5 min. Then, glass powder was packed with a 5 s

centrifugation and supernatant was discarded, 1 mL ethanol wash buffer was added, glass powder was suspend with a pipet tip and glass powder was centrifugated again with a brief centrifugation. Ethanol was discarded and tubes were dried on a heat block (55 ºC, 10 min). Glass powder was suspended in sterile distilled water (100 μL) and heated for 1 min (55 ºC). Finally, glass powder was packed and eluted DNA was transferred to a clean tube and stored at -20º C. Aliquots were used to check DNA quantity and quality.

Amplification and sequencing.

DNA sequences from the B fragment were obtained by cycle sequencing of PCR-amplified DNA. PCR reactions were performed in 50 μL final volume containing 2 μL template DNA, 5 μL of 10X PCR buffer (10 mM Tris/HCl pH 8.0, 50 mM KCl, 1.5-2.0 mM $MgCl_2$), 4 μL of dNTP mix (1.25 mM each dATP, dCTP, dGTP, and dTTP), 10 pmol each of the opposing amplification primers, 0.5 μL *Taq* polymerase (Promega, Madison, WI). The reactions were run in a MJ Research PTC-200 thermocycler, using a touchdown PCR procedure (Rehner and Buckley, 2005). Primers B5.1 and B3.1R were used for spanning B fragment (Table 3).

Before to separate the PCR products by electrophoresis, the PCR volumes were reduced in a Speed-Vac (2 h, highest desiccation rate). Remnant volume was eliminated with a brief spin. DNA was resuspended in 1X low EDTA (0.1 mM) TAE electrophoresis buffer to which has been added 1/10 volume loading buffer. PCR products were loaded onto 1.5% NuSieve agarose gels (Bio-Whitakker, Rockland, Maine), stained with ethidium bromide. After the run, the gel was cooled (15 min, 5 ºC). The amplicons were visualized by exposing the gel to UV light and excised using a broad-nosed scalpel blade. Gel slices were frozen (-20 ºC) until sequencing. At this point, gel slices were thawed and centrifuged in a microcentrifuge (20000 x g, 10 min) to compress the gel slice and extrudes DNA.

The sequencing reactions were performed with ABI BigDye 2.0 (Applied Systems, Foster City, CA) with a total volume of 5 μl, which included 0.5 μl BigDye diluted in 1,5 μl dilution buffer, 3 pmol primer, 100 ng gel-purified PCR template. Cycle sequencing was performed in 96-well microtiter plates according to the manufacturer's instructions. The

products were precipitated adding ethanol. The sequencing reactions were suspended in deionized formamide, heat denatured and run on an ABI 3100 Genetic Analyzer (Applied Systems, Foster City, CA).

The resulting chromatographs were imported into Sequencher 4.1 (Gene Codes Corp., Ann Arbor, Michigan) for visual inspection and editing. Multiple sequence alignments were constructed with the MegAlign module of DNASTAR 5 (Lasergene, Madison, WI).

Data analysis.

Phylogenetic approaches.

The ninety seven sequences were aligned using the Clustal W module of MEGA version 3.1 (Kumar et al., 2004) and collapsed into haplotypes using the DNASP 4.10 software (Rozas et al., 2003). The phylogenetic relationships among the whole sequences and the extracted haplotypes were evaluated by using neighbour-joining (NJ), maximum parsimony (MP) and minimum evolution (ME) methods. All analyses were conducted using the Phylogeny module of MEGA version 3.1 (Kumar et al., 2004).

The NJ tree was constructed from the matrix of pairwise p distances. This distance measure was chosen because it has low variance and then it is suitable to deal with low variation sequences (Nei and Kumar, 2000). Both MP and ME trees were constructed using unweighted data. Bootstrapping values (600 pseudoreplicates) were used to determine nodal support. The trees were rooted using a sequence obtained from the rice blast fungus *Magnaporthe rosea*, retrieved from Genbank (XM_368948, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=Nucleotide&list_uids=39975114&dopt=GenBank). We considered gapped positions as unreliable characters and excluded them from further analysis (Swofford et al, 1996). To further analyze the genetic relatedness of haplotypes, we constructed a haplotype network using the median-joining network method as implemented in Network software (Bandelt et al., 1999; www.fluxus-engineering.com).

Analyses of demographic history.

The mismatch analysis (the distribution of the observed number of differences between pairs of haplotypes) was adopted to examine the demographic history of the species

(Schneider and Excoffier, 1999; Althoff and Pellmyr, 2002). The resulting distribution was compared against a simulated distribution assuming the constant growth model as implemented in DNASP (Rozas et., 2003). A unimodal distribution is expected if the lineages have undergone a recent bottleneck or population expansion, while a multimodal or ragged distribution is expected for a lineage whose populations are at demographic equilibrium (Althoff and Pellmyr, 2002).

In addition, we used Tajima's D to examine further the historical demography of *B. bassiana sensu lato* (Althoff and Pellmyr, 2002). This test for selective neutrality can be used to examine demography: a significant negative value indicate a deviation from the expectations of the mutation-drift equilibrium and can indicate population expansion, while a positive value is expected under population subdivision. Under neutrality the number of nucleotides differences between sequences from a random sample should be equal to the number of differences between the polymorphic sites only, but population expansions can cause negative departures of Tajima's D from zero.

The molecular diversity of the data set was calculated using the Arlequin 3.01 software (Schneider et al., 2000), including the average number of nucleotides per sites (nucleotide diversity π; Nei, 1987) and haplotype diversity. These parameters were calculated because centers of origin should be more diverse than more recently founded populations (Althoff and Pellmyr, 2002).

Analyses of population structure.

Hierarchical structuring of genetic variation was determined using analysis of molecular variance (AMOVA, Excoffier et al., 1992) as implemented in the Arlequin 3.01 software (Schneider et al., 2000). This analysis computes fixation indices ϕ_{ST} analogous to Wright's (1951) F statistics to divide the variance into the different components. The significance of the *a priori* geographical groupings was tested by bootstrapping (1023 permutations). The molecular distances between pairs of sequences necessary to run AMOVA were computed under the Kimura 2P model.

The pairwise genetic distance matrixes were calculated using the Arlequin 3.01 software package (Schneider et al., 2000) to additionally compare the putative populations.

Results.

Phylogenetic analyses.

The neighbour-joining (NJ), maximum parsimony (MP) and minimum evolution (ME) trees showed no major differences on the inferred phylogenies and consequently only the NJ tree is shown in Figure 1. The only relevant difference between trees was the sister position of the isolate B900 (haplotype 11). The branching pattern suggests a deep division for the *B. bassiana* s.l. in Chile. The two large clusters include some smaller but well supported clusters. When the phylogenetic and geographic data were crossed (Figure 1), the phylogenetic relatedness between isolates did not agree with their geographic relatedness, except for a small cluster which exclusively comprised isolates collected at Eastern Island. As expected, the haplotype tree shows a condensed sight of the findings (Figure 2).

The Figure 3 shows the haplotype network constructed from the most parsimonous trees supported by the dataset. Remarkly, the network grouped the haplotypes into two large clusters, with the haplotype 11 in a intermediate position. The most extended haplotype (H1) has not a interior position in the network as expected if this haplotype was the ancestral state.

The segment had a nucleotide composition of A=0.24, C=0.28, G=0.27 and T=0.21, with G+C content = 55%. The 97 sequences gave 20 different haplotypes, which included 282 variable (19%) and 218 parsimony informative (15%) sites. The overall nucleotide diversity was 0.035.

Demographic analyses.

The mismatch distributions for all pairwise combinations of the individuals for each population are shown in the Figure 4, except for Tarapacá, which was revealed fixed for the haplotype 1 and consequently the mismatch distribution can not be calculated. All the six populations had bimodal or multimodal distributions which did not conformed to the expected distribution from a model of constant growth population expansion.

All the putative populations showed Tajima's D values that did not significantly departure from zero, except for Aysén (Table 4). This finding did not support the idea that populations passed through expansion.

The haplotype 1 (H1) accounted for 49 sequences (50.5%), followed by haplotypes 3 and 17 with 11 and 6 sequences (11.3 and 6.1%, respectively). Eleven haplotypes were unique. The most abundant haplotype H1 was found accross the country, but other were restricted to one population. In special, H17 (n=6) and H18 (n=1) were present only at the Eastern Island population, while H8 (n=4) was unique to Los Lagos population. Sixteen haplotypes were found only in the mainland populations, although this result would be interpreted with caution because many haplotypes were unique and a sampling effect must not be dismissed. From Figure 5, some likely patterns arise. First, the haplotype 1 was present in all the sampled populations, from the Northernmost part of the country to the South, including Eastern Island, and it may be the most ancestral because of its wide range. However, an ancestral status for haplotype 1 is not supported by its position in the haplotype network (Figure 3).

Second, Eastern Island had two haplotypes not found in its mainland counterparts. Finally, two populations (Maule-Biobío and Los Lagos) seem to be more diverse than the rest of the populations.

Population structure analyses.

Little but significant ($p<0.01$) support for the proposed grouping came from AMOVA analysis (Table 5). The variation between populations accounted for only the 8.8% of the total variation. Nevertheless, pairwise comparisons showed that several populations were distinguishable into the sample. For example, Eastern Island was significantly different from all mainland populations, except Magallanes. The Tarapacá population, fixed for haplotype 1, additionally was different from Central Chile, Maule-Biobío and Los Lagos, but not from the Southernmost populations Aysén and Magallanes. Finally, Aysén was significatly different from Central Chile and Maule-Biobío.

Discussion.

In our opinion, the adopted combination of techniques (Althoff and Pellmyr, 2002), is well suited to deal with topics such as whose studied in this research. Some populations are far apart (even thousands of kilometers) enough to allow hypothetical vicariant events to occur. However, when the B fragment phylogeny was overlain on the current geographic distribution of the isolates, no clear pattern was observed. On the other hand, the AMOVA analysis do detect a small but significant population structuring, which could be resulted from more recent events, including human-mediated dispersal of the fungus through movement of crop plants and soil associated to them.

The demographic analyses proved to be complementary to the phylogenetic approach. As noted by Althoff and Pellmyr (2002), when the phylogenetic approach reveals a geographic structure, demographic approach generally agrees. But when the phylogenetic approach fails to detect such a structure, especially if recent demographic changes has occurred, then the demographic approaches are useful in that temporal scale. In this particular fungal population, it seems to be the case and reinforces the use of a combined approach (Bernatchez, 2001; Althoff and Pellmyr, 2002).

We expected a clear differentiation between Eastern Island and continental Chile, based in the brief period in which the island has been influenced by the continent (Eastern Island was anexed to Chile at 1888). The division was clear when AMOVA and pairwise comparisons were used, but the phylogenetic signal for this division was weak. Eight Eastern Island's isolates were grouped closely, but other six isolates were sparsed in a large cluster including isolates from all remaining populations.

Early studies conducted on *B. bassiana* did not agree on the putative genetic relatedness and geographic origin. While some authors reported that they are correlated (Wang et al., 2003; Yang et al., 2005), others did not support this conclusion (Aquino de Muro et al., 2003; Aquino de Muro et al., 2005; Berretta et al., 1998; Coates et al., 2002). The studies differ largely on sample size, molecular methods and statistical analysis, being hard to properly compare each other. Others authors have suggested that genetic relatedness is

strongly influenced by insect host (RAPD and RFLP, Maurer et al., 1997; RFLP, Viaud et al., 1996; ITS, Neuveglise et al., 1994). Again, this conclusion is refuted by other studies (ITS sequencing and RFLP, Coates et al., 2002; RAPD, Castrillo et al., 2004; Berreta et al., 1998; Urtz and Rice, 1997).

The fungus *Beauveria bassiana* has been studied for more than 150 years and an asexual stage has been assumed for the most part of that period. In recent years, molecular studies have identified *Beauveria* as the asexual stage of *Cordyceps* spp., which reproduces by a sexual cycle. Despite of the evidence for this linking, it seems that *Beauveria* spp. is asexual for the most of its lifecycle. Under this condition, some genetic recombination still is possible through parasexual cycle, during which vegetatively compatible hyphae fuse to exchange genetic material (Paccola-Meirelles and Azevedo, 1991). However, the parasexual cycle in this species is controled by a strict recognition system and many different vegetative compatibility groups can be identified in a sample (Castrillo et al., 2004). Consequently, the genetic exchange seems quite restricted in *B. bassiana* s.l.

Another plausible explanation for the phylogenetic pattern showed in the Figure 1 is related what Rehner and Buckley (2005) proposed as cryptic speciation into the morphologically defined *B. bassiana*. These authors reported that globally collected *B. bassiana* would be paraphyletic according to a two nuclear genes phylogeny, the elongation factor 1 alpha (EF1-α) and the ribosomal internal transcribed spacer (ITS). The combined analysis divided the previously identified *B. bassiana* isolates in two clades separated by *B. brongniartii*. The B locus phylogeny mostly resembles this pattern and consequently a subset of Chilean isolates, representative of the major B fragment clades, was additionally sequenced for EF1-α (Devotto and Rehner, unpub. data). One isolate was more related to *B. amorpha* than *B. bassiana*, while one strain was grouped in one of the *B. bassiana* clusters and the remaining nine isolates were included in the paraphyletic *B. bassiana* cluster. It will be necessary to include a larger Chilean sample in this proposed phylogeny to test if the Chilean population mirrors the pattern detected for the *Beauveria* genus.

Potential for fingerprinting.

The resolutive power of the sequenced region was comparable to other molecular tools used for *B. bassiana* fingerprinting. A sample of 96 isolates was separated into 24 genotypes when Coates et al., (2002) performed PCR-RFLP on it, a level of resolution close to the 20 genotypes found in our 97 isolates set. Culture methods like vegetative compatibility grouping also have shown high polymorfism in this fungus (Castrillo et al., 2004; Couteaudier and Viaud, 1997), but they are more time and labour-consuming than sequencing.

Both anonymous DNA (RAPD, Bidochka et al., 1994; Maurer et al 1997; Castrillo et al., 2003; minisatellite, Coates et al., 2001) and non anonymous DNA (ITS region, Aquino de Muro et al., 2003; Neuveglise et al., 1994, 1997; Glare and Inwood, 1998; 28s rDNA region, Wang et al 2002; telomere, Couteaudier and Viaud, 1997; nuclear small subunit rRNA (nuSSU rRNA) introns, Coates et al., 2002b) have been targeted for fingerprintig. When a global *B. bassiana* sample was assessed, the ITS region was less variable than the gene elongation factor 1 alpha EF1-α (Rehner and Buckley, 2005) and the B fragment has shown to be more variant than EF1-α (Rehner, unpubl. data).

Castrillo et al., (2003) developed SCAR markers from unique RAPD bands, which were able to distinguish one isolate of particular interest from other indigenous *B. bassiana*. This method has great potential because was useful to detect minute amount of target DNA directly from soil, but it can not be applied to several isolates simultanously. In this case, SCARs for each single isolate must be designed. The B fragment sequencing do detect several isolates at the same time, although with a relatively lower power resolution than multiple SCARs.

Conclusions.

The survey of B fragment variation within and among populations of *B. bassiana* throughout a large part of its geographical range in Chile has showed that many genotypes exist into the species. The phylogenetic analysis yielded weak evidence for a correlation between genetic variation and geographical origin. However, population structure tests such as AMOVA and genetic distance pairwise comparisons of putative populations

supported a geographical sub-division for the fungus, suggesting that the events causing this pattern occurred in the recent past. Demographic history of populations showed relatively stable populations through time. The B fragment showed to be a good candidate for strain fingeprinting, at least for a significant proportion of the sample.

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Table 1. Environmental data for the defined populations included in this study (based on Novoa and Villaseca, 1989; Papadakis, 1970).

Population	Number of isolates	Rainfall (mm per year)	Temperature (mean average year)	Climate
Tarapacá	8	0.2	19	Desertic
Central Chile	6	436	15	Mediterranean
Maule-Biobío	41	1025	13	Temperate Mediterranean
Los Lagos	15	1383	11	Temperate
Aysén	10	2973	7	Marine
Magallanes	3	416	7	Marine wet Patagonic
Eastern Island	14	1091	20	Tropical

Table 2. List of isolates included in the study. They are hold at the Entomopathogenic Organisms Collection, Instituto de Investigaciones Agropecuarias (INIA), Regional Research Centre Quilamapu, Chillán, Chile (Dr. Andrés France, afrance@inia.cl).

Isolate code	Locality	Latitude	Longitude	Population
B299	Lago Chungará	18º 15'	69º 12'	Tarapacá
B303	Valle Lluta	18º 26'	70º 04'	Tarapacá
B306	Río Chaca, Arica	18º 18'	70º 11'	Tarapacá
B308	Putre	18º 12'	69º 13'	Tarapacá
B323	Río Chaca, Arica	18º 18'	70º 11'	Tarapacá
B294	Lago Chungará	18º 15'	69º 12'	Tarapacá
B300	Valle Lluta	18º 20'	69º 44'	Tarapacá
B310	Putre	18º 12'	69º 13'	Tarapacá
B329	Puente El Teniente, Chagual	30º 41'	71º 34'	Central Chile
B334	El Tambo, Vicuña	30º 10'	71º 13'	Central Chile
B984	Quillota	32º 12'	71º 10'	Central Chile
B330	El Tambo, Vicuña	30º 00'	70º 51'	Central Chile
B333	Las Cardas	30º 10'	71º 13'	Central Chile
B927	Placilla	33º 4'	71º 34'	Central Chile
B889	Rano Kau	27º 06'	109º 21'	Eastern Island
B890a	Rano Kau	27º 06'	109º 21'	Eastern Island
B892b	Rano Kau	27º 06'	109º 21'	Eastern Island
B893	Rano kau	27º 06'	109º 21'	Eastern Island
B894	Orongo	27º 06'	109º 21'	Eastern Island
B897	Temiro Oone	27º 06'	109º 21'	Eastern Island
B899	Anakena	27º 06'	109º 21'	Eastern Island
B900	Motu Kao	27º 06'	109º 21'	Eastern Island
B902	Anakena.	27º 06'	109º 21'	Eastern Island

B907	Poike	27º 06'	109º 21'	Eastern Island
B910	Ahu Hanga Poukura	27º 06'	109º 21'	Eastern Island
B913	Hangaroa	27º 06'	109º 21'	Eastern Island
B915	Rano Ranaku	27º 06'	109º 21'	Eastern Island
B923	Maunga puka	27º 06'	109º 21'	Eastern Island
B255	Cañete	37º 47'	73º 20'	Maule-Biobío
B273	Cañete	37º 47'	73º 20'	Maule-Biobío
B314	Santa Lucía, Alto Yungay	37º 06'	72º 00'	Maule-Biobío
B325	Santa Lucía, Alto Yungay	37º 06'	72º 00'	Maule-Biobío
B342	Tirúa, Cañete	38º 20'	73º 20'	Maule-Biobío
B366	Entrada Constitución	35º 19'	72º 25'	Maule-Biobío
B368	Río Maule Constitución	35º 19'	72º 25'	Maule-Biobío
B378	Junquillar, Constitución	35º 19'	72º 25'	Maule-Biobío
B392	Cañete	37º 47'	73º 20'	Maule-Biobío
B408	Cañete	40º 38'	72º 10'	Maule-Biobío
B437	Huape	36º 37'	72º 10'	Maule-Biobío
B501	Bulnes	36º 43'	72º 16'	Maule-Biobío
B502	Bulnes	36º 43'	72º 16'	Maule-Biobío
B505	Portezuelo	36º 30'	72º 31'	Maule-Biobío
B507	Angol	37º 04'	72º 07'	Maule-Biobío
B508	Angol	37º 04'	72º 07'	Maule-Biobío
B509	Angol	37º 04'	72º 07'	Maule-Biobío
B513	Cañete	37º 47'	73º 20'	Maule-Biobío
B515	Molina	37º 38'	71º 00'	Maule-Biobío
B518a	Molina	37º 38'	71º 00'	Maule-Biobío
B518b	Molina	37º 38'	71º 00'	Maule-Biobío
B532	Santa Bárbara	37º 38'	71º 00'	Maule-Biobío
B640	Liucura, Tucapel	37º 15'	71º 55'	Maule-Biobío

B837	Pinto	36º 43'	71º 53'	Maule-Biobío
B941	Coihueco	36º 37'	71º 53'	Maule-Biobío
B467	Pinto	36º 55'	71º 26'	Maule-Biobío
B360	Pinto	36º 41'	71º 53'	Maule-Biobío
B389	Puente Culenes, Curepto	35º 05'	72º 01'	Maule-Biobío
B390	Pelluhue	35º 49'	72º 04'	Maule-Biobío
B482	Cañete	37º 47'	73º 20'	Maule-Biobío
B484	Cañete	37º 47'	73º 20'	Maule-Biobío
B506	Portezuelo	36º 30'	72º 31'	Maule-Biobío
B797	Pencahue	35º 20'	71º 55'	Maule-Biobío
B824	El Carmen	36º 53'	71º 55'	Maule-Biobío
B833	Paso Alejo, Coihueco	36º 38'	71º 53'	Maule-Biobío
B858	Portezuelo	36º 29’	72º 24'	Maule-Biobío
B859	Quillón	36º 43'	72º 28'	Maule-Biobío
B864	Villa Alegre	35º 38'	71º 44'	Maule-Biobío
B934	Paso Alejo, Coihueco	37º 44'	71º 40'	Maule-Biobío
B983	Chillán	36º 36'	72º 06'	Maule-Biobío
B274	Cañete	37º 47'	73º 20'	Maule-Biobío
B499	La Unión	40º 15'	73º 06'	Los Lagos
B72	Osorno	40º 28'	73º 04'	Los Lagos
B179	Pumanzano	41º 53'	73º 45'	Los Lagos
B321	Puyehue, Osorno	40º 38'	72º 10'	Los Lagos
B428	Puyehue, Osorno	40º 38'	72º 10'	Los Lagos
B492	Trosquilmo	40º 35'	73º 24'	Los Lagos
B494	Río Tea, Osorno	40º 35'	73º 28'	Los Lagos
B495	Río Tea, Osorno	40º 35'	73º 28'	Los Lagos
B496	Río Tea, Osorno	40º 35'	73º 28'	Los Lagos
B504	Frutillar	41º 05'	73º 07'	Los Lagos

B606	Lago Icalma	38º 50'	71º 20'	Los Lagos
B931	Remehue, Osorno	40º 29'	73º 05'	Los Lagos
B491	Corral	39º 52'	73º 24'	Los Lagos
B926	Río Bueno	40º 18'	72º 57'	Los Lagos
B937	Osorno	40º 32'	73º 34'	Los Lagos
B456	Balmaceda Airport	45º 54'	71º 43'	Aysén
B460	Balmaceda Airport	45º 54'	71º 43'	Aysén
B771	Balmaceda	45º 54'	71º 45'	Aysén
B774	Puerto Cisnes	44º 40'	72º 35'	Aysén
B787a	Reserva Nacional Río Simpson	45º 33'	72º 25'	Aysén
B765	Puerto Ibáñez, Cerro Castillo	46º 08'	72º oo’	Aysén
B776	Coyhaique	45º 18'	71º 57'	Aysén
B787b	Reserva Nacional Río Simpson	36º 52'	71º 57'	Aysén
B806	Villa Amengual	44º 45'	72º 18'	Aysén
B822	Río Cisnes	44º 44'	72º 35'	Aysén
B472	Valle Los Castores, Tierra del Fuego	53º 00'	70º 00'	Magallanes
B473b	Tierra del Fuego	53º 00'	70º 00'	Magallanes
B795	Punta Arenas	53º 00’	70º 53'	Magallanes

Table 3. Primers designed by S.A. Rehner (Insect Biocontrol Laboratory, USDA-ARS, Beltsville, Maryland) to amplify B fragment segment.

	PRIMER NAME	**SEQUENCE (5' – 3')**
Forward	B5.1F	CGACCCGGCCAACTACTTTGA
	B22U	GTCGCAGCCAGAGCAACT
	BFint	GTTCCTTGCCCTCGGTAATGAA
Reverse	BRint	AGCATATCGGGCATGACTGA
	B822L	AGATTCGCAACGTCAACTT
	B3.1R	GTCTTCCAGTACCACTACGCC

Figure 1. NJ tree of the B fragment phylogeny inferred for *B. bassiana*. Bootstrap values above nodes indicate support for branches (500 pseudorepliques). The whole figure was halved because its size and miniaturized (above, left) to show the overall pattern.

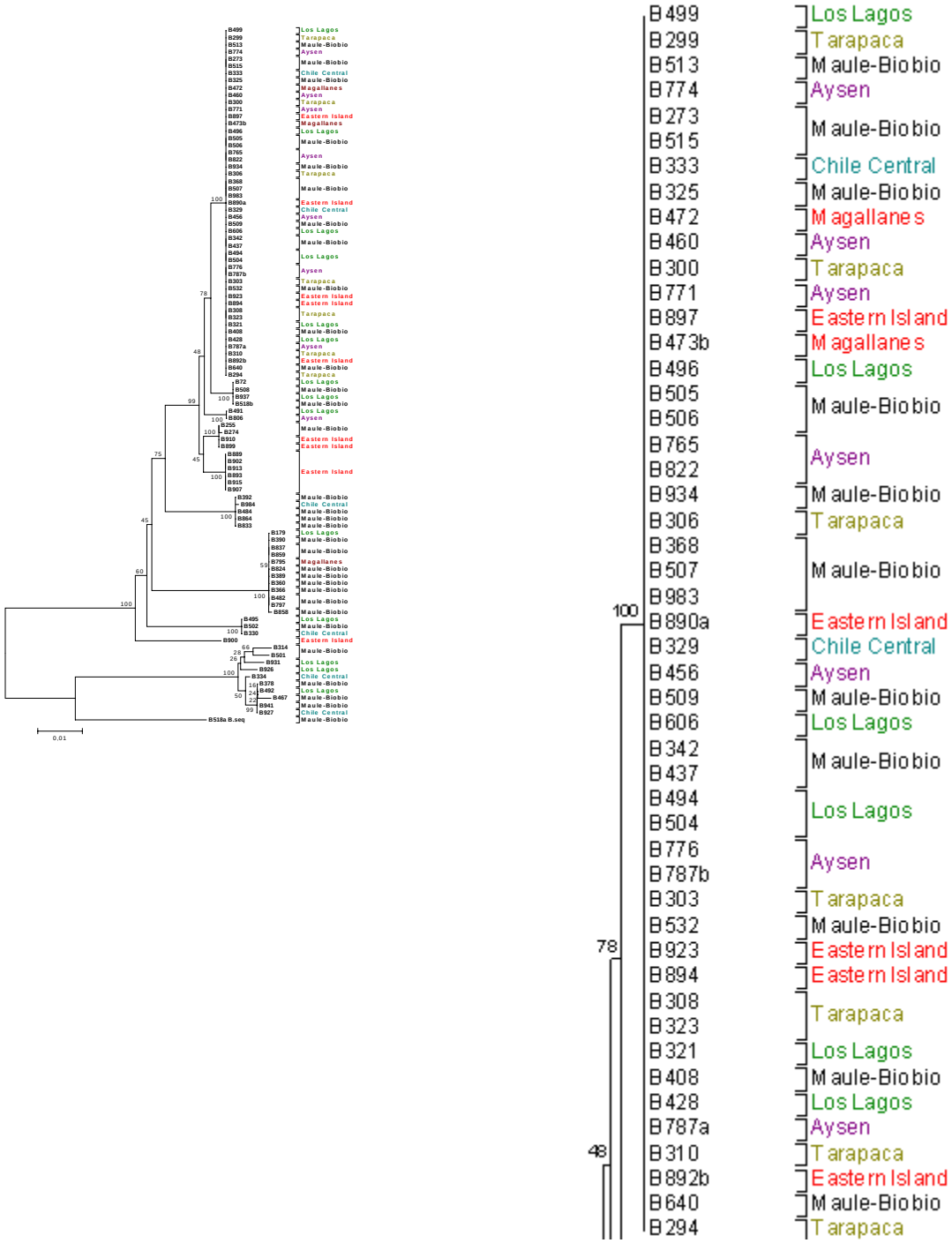

Figure 1 (continued).

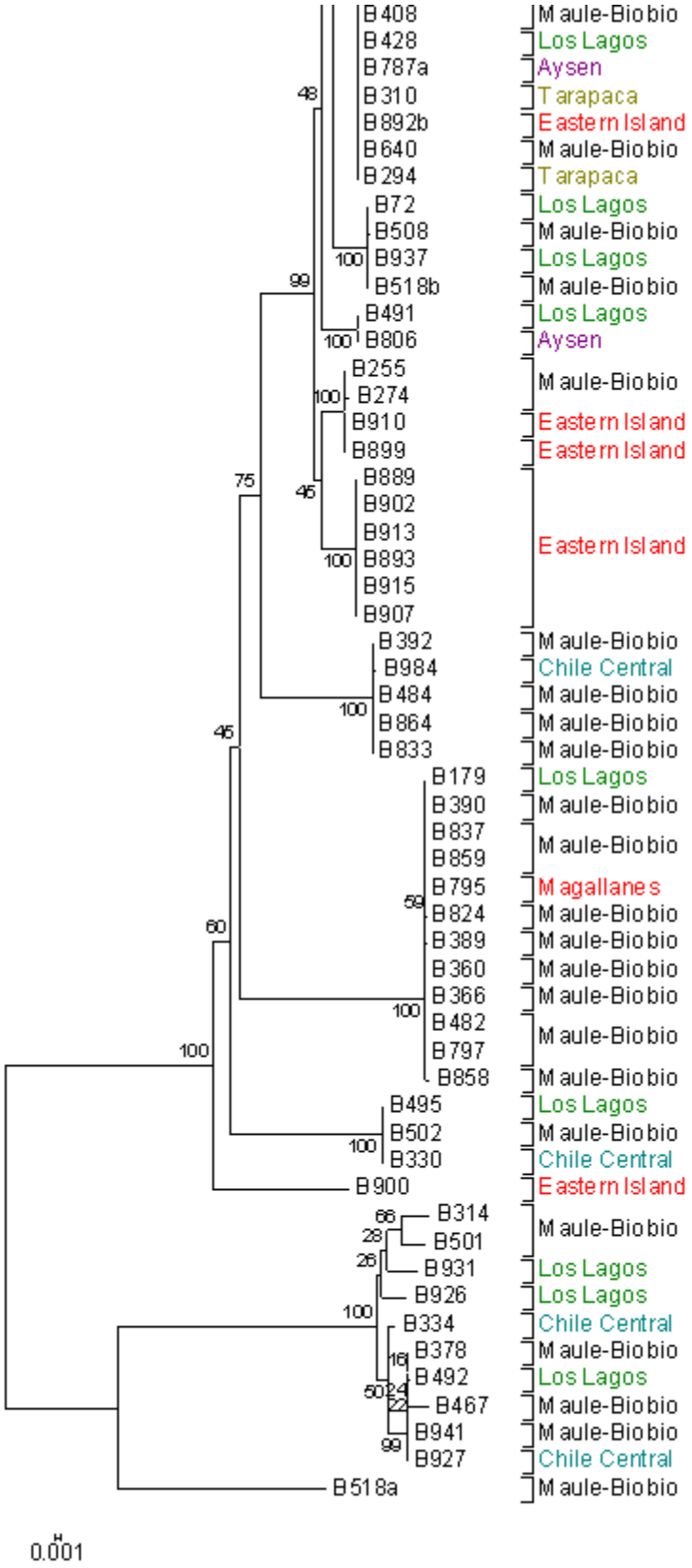

Figure 2. Haplotype tree inferred from 97 *B. bassiana* isolates sampled in Chile. Number of isolates included in each haplotype is shown in brackets. Haplotype 21 corresponds to *Magnaporthe rosea*.

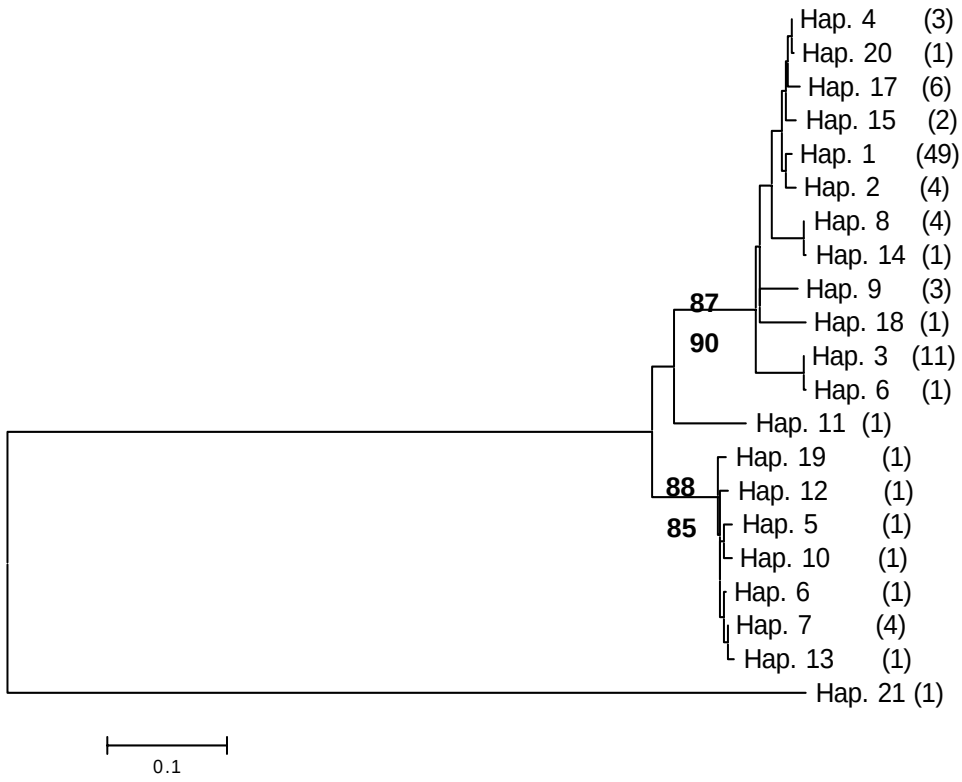

Figure 3. Median-joining network for B fragment haplotypes. The size of the circles is proportional to the frequency of the represented haplotype. Black dots represent the median vectors may be hypothetical missing or unsampled ancestral haplotypes. The haplotype corresponding to B931 isolate is rounded in red.

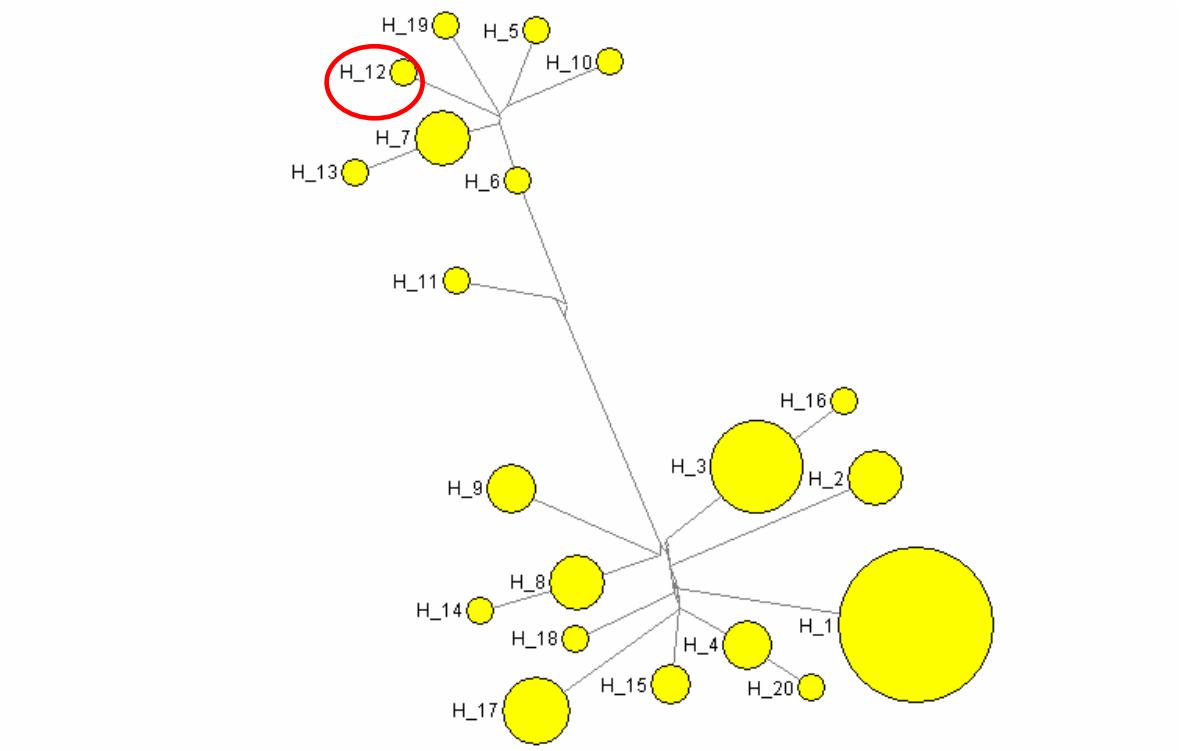

Figure 4. Mismatch distributions of *B. bassiana* from six sampled populations in Chile. Straight line represents the expected distribution assuming constant growth population model. Dash lines with diamonds represent the observed distribution of pairwise differences.

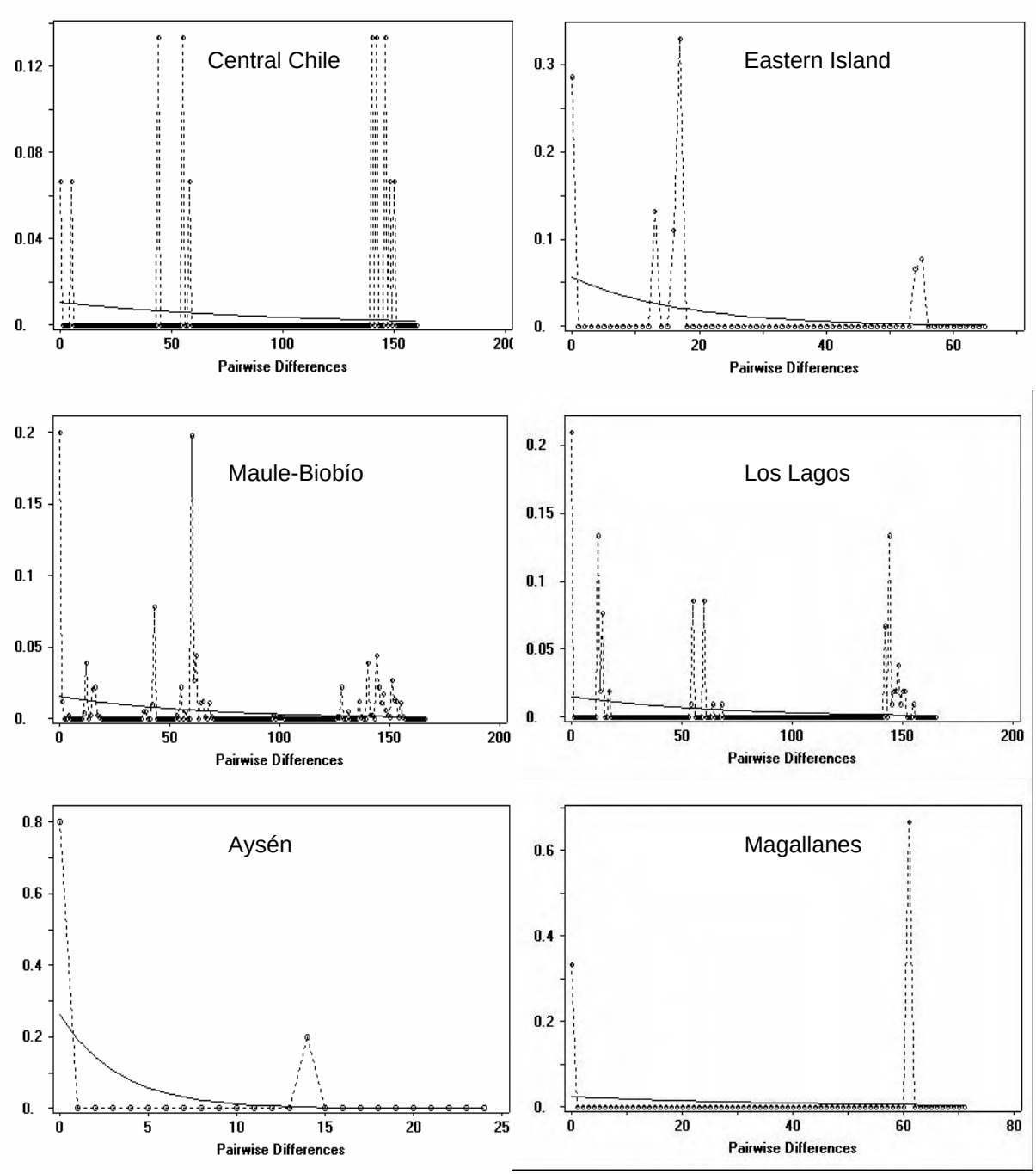

Table 4. Estimates of haplotype and nucleotide diversity for different populations of *Beauveria bassiana* in Chile.

Population	Haplotype diversity	Nucleotide diversity	N	Haplotype number	Tajima's D (significance)
Tarapacá	0.000 ± 0.000	0.000 ± 0.000	8	1	
Magallanes	0.667 ± 0.314	0.143 ± 0.108	3	2	0.000 (p=0.91)
Aysén	0.200 ± 0.154	0.010 ± 0.007	10	2	-1.989 (p<0.00)
Central Chile	0.933 ± 0.122	0.337 ± 0.196	6	5	1.035 (p=0.84)
Maule-Biobío	0.800 ± 0.049	0.227 ± 0.111	41	13	0.235 (p=0.63)
Los Lagos	0.791 ± 0.105	0.231 ± 0.119	15	8	0.011 (p=0.55)
Eastern Island	0.714 ± 0.078	0.060 ± 0.032	14	4	-0.937 (p=0.18)
Overall	0.727 ± 0.047	0.035 ± 0.004	97	20	-0.487 (p=0.10)

Figure 5. Haplotype diversity in the seven sampled populations of *B. bassiana*. Hap. 1 = red; hap. 2 = pale blue; hap. 3 = green; hap. 7 = blue; hap. 8 = pink; hap. 17 = yellow; and minor haplotypes = orange.

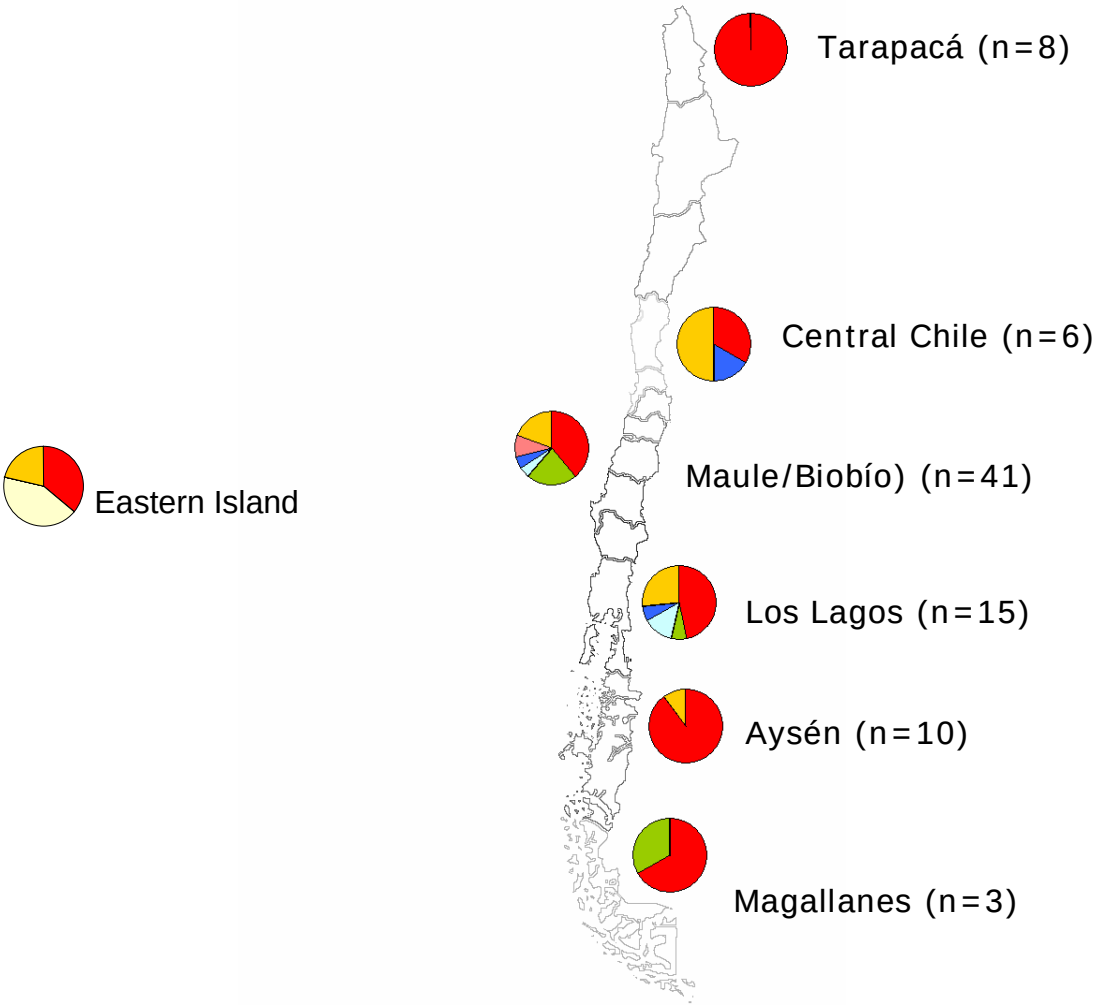

Table 5. Analyses of molecular variance (AMOVA) for B fragment haplotypes in seven *B. bassiana* populations sampled from Chile, conducted among and within all populations.

Source of variation	df	Sum of squares	Variance component	% total variance	P (1023 permutations)
Among populations	6	303.96	2.2483	8.82	0.0088
Within populations	90	2091.58	23.2398	91.18	
Total	96	2395.54	25.4881		
Fixation index	Fst:	0.088			

Table 6. Analyses of molecular variance (AMOVA) for B fragment haplotypes in *B. bassiana* sampled from Chile. The Eastern Island was contrasted with the mainland group, which included the six remaining populations.

Source of variation	df	Sum of squares	Variance component	% total variance	Fixation indices and significance test (1023 permutations)
Among groups (Va) F_{CT}	1	169.985	3.30029	5.08	0.05081 ($p \leq 0.111$)
Among populations within groups (Vb) F_{SC}	5	413.120	1.99602	3.07	0.03237 ($p \geq 0.077$)
Within populations (Vc) F_{ST}	90	5369.634	59.66260	91.85	0.08153 ($p \geq 0.286$)
Total	96	5952.740	64.66260		

St: similarity of any 2 sequences from the same population in relation to the similarity of pair of sequences drawn from all the samples.

Ct: similarity of any 2 sequences from the same group of localities relative to any 2 sequences from all the sequences.

Sc: similarity of any 2 sequences from the same locality in relation to the similarity of any 2 sequences from the same group of localities.

Table 7. Pairwise differentiation estimates among populations based on B fragment haplotype data, showing pairwise estimates of Fst (from haplotype frequencies). Asterisks (*) indicate Fst values that are significantly different from zero ($p<0.05$; 110 permutations).

		Tarapacá	Magallanes	Aysén	Central Chile	Maule-Biobío	Los Lagos	Eastern Island
1	Tarapacá	0						
2	Magallanes	0.342	0					
3	Aysén	-0.024	0.084	0				
4	Central Chile	0.362 *	-0.059	0.257 *	0			
5	Maule-Biobío	0.199 *	-0.124	0.154 *	0.001	0		
6	Los Lagos	0.178 *	-0.114	0.108	-0.040	-0.005	0	
7	Eastern Island	0.364 *	0.084	0.298 *	0.081	0.112 *	0.097 *	0

Table 8. Pairwise differentiation estimates among populations based on B fragment haplotype data, showing pairwise estimates of Fst (from pairwise distances). Asterisks (*) indicate Fst values that are significantly different from zero ($p<0.05$; 110 permutations).

		Tarapacá	Magallanes	Aysén	Central Chile	Maule-Biobío	Los Lagos	Eastern Island
1	Desertic	0						
2	Sub_Antarctic	0.342	0					
3	Cold	-0.024	0.313	0				
4	Northern_Med	0.321 *	0.012	0.347 *	0			
5	Southern_Med	0.118 *	-0.136	0.125 *	0.022	0		
6	Temperate	0.095	-0.085	0.105	-0.044	-0.005	0	
7	Eastern Island	0.298 *	0.198	0.266 *	0.309 *	0.130 *	0.134 *	0

2.- CAPÍTULO SEGUNDO: ANÁLISIS DE LOS EFECTOS NO DESEADOS A NIVEL DE TAXA INDIVIDUALES.

Este artículo puede ser consultado bajo el título:

Devotto L, R. Carrillo, E. Cisternas and M. Gerding, in press. Conservation biological control of soil surface predators (carabid beetles and spiders) and compatibility with *Beauveria bassiana* spores and lambda-cyhalothrin in a Southern Chilean pasture. Pedobiologia.

CONSERVATION BIOLOGICAL CONTROL OF SOIL SURFACE PREDATORS (CARABID BEETLES AND SPIDERS) AND COMPATIBILITY WITH *B. bassiana* SPORES AND LAMBDA-CYHALOTHRIN IN A SOUTHERN CHILEAN PASTURE.

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Running title: Inundative release of *B. bassiana* and conservation of surface predators.

Summary.

The effects on generalist predators of the insecticide lambda-cyhalothrin and a new biopesticide based on *Beauveria bassiana* spores were studied in the 2003 growing season (October to December, Southern spring) in Valdivia, Chile. Both pesticides are targeted against larvae of *Dalaca* spp. (Lepidoptera: Hepialidae), a complex formed by *Dalaca chiliensis*, *Dalaca pallens* and *Dalaca variabilis*. Sampling revealed an assemblage of 11 carabid species and two spider families (Lycosidae and Gnaphosidae). In order of abundance, the carabid species detected were *Ferionomorpha nebroides* (45%), *Allendia chilensis* (20%), *Argutoridius chilensis* (13%), *Ferionomorpha aerea* (11%) and *Ceroglossus chilensis* (6%). Other six species accounted by less than 1% each (*Metius flavipes*, *Mimodromites cyaneus*, *Parhypates* sp., *Trechisibus angularis*, *Calosoma vagans* and *Trirammatus unistriatus*). Spider families were almost equally represented. In the first two sampling dates, low numbers of predators precluded to detect effects of treatments, except for *F. nebroides*. Activity of predators increased in time and negative effects of lambda-cyhalothrin were detected on *F. nebroides*, *F. aerea*, Lycosidae and Gnaphosidae. Most of decreases were detected by day 30 after spraying. Differences did vanish by day 60 on spiders, but they persisted on some carabids. Inundative release of *B. bassiana* spores did not affected any taxa, being a good potential alternative to the broad-spectrum insecticide, which disrupted the generalist predator assemblage severely.

Keywords.

Inundative biological control; non-target; lambda-cyhalothrin; Carabidae; Lycosidae; Gnaphosidae.

Introduction.

In last decades, modern agriculture has implied ecosystem simplification, emphasis on yield and intensive inputs, including pesticides (Banks, 2004). Endemic natural enemies of insect pests represent a fundamental resource for maintaining pest population levels under economic thresholds and maximizing their contribution to integrated pest management (IPM) programs requires a detailed knowledge of their interactions with the target pest (Furlong et al., 2004), population dynamics and response to disturbances.

Conservation biological control has been reinforced because of increasing awareness on threats posed by alien biological control agents to native non-target species (Banks, 2004). Ground beetles (Coleoptera: Carabidae) are one of the most common families of surface-active arthropods in agricultural systems (Lövei and Sunderland, 1996; Cole et al., 2002), while spiders are increasingly recognized as valuable biocontrol agents (Greenstone, 1999). Several experimental studies have repeatedly demonstrated positive effects of generalist predators on several crops, including corn (Clark et al., 1994), wheat (Dennis and Wratten, 1991) and oat (Helenius, 1990). Spiders meet several desirable characteristics as biological control agents: they are relatively long lived, resistant to starvation and desiccation, good dispersers and colonizers and predaceous at all stages of development (Greenstone, 1999). On the other hand, carabids are abundant year roundly and they do not depend on only one prey species. Many pesticides, including lambda-cyhalothrin, induce significant levels of mortality in carabids and spiders (Epstein et al., 2000) thereby impacting their potential to maintain pests in check.

In Southern Chile, the pasture yield is severely affected by the activity of *Dalaca pallens* Blanchard, *Dalaca chiliensis* (Viette) and *Dalaca variabilis* larvae (Viette) (Lepidoptera: Hepialidae). In general, *D. pallens* is the most abundant species in pastures and >200.000 ha per year are sprayed to control it. Insect growth regulators are used against early larval stages of the pest, while broad spectrum pyrethroids such as lambda-cyhalothrin are sprayed in Autumn to control neonate larvae and in Spring to control mature larvae. Previous studies have shown that the fungus *Beauveria bassiana* applied at 10^{12} spores per ha has 80-100% of the lambda-cyhalothrin efficacy at controlling *D. pallens* larvae (Cisternas, 2003).

Adverse effects of pesticides on ground beetles have been widely documented in the Northern Hemisphere, but little is known about the response of other populations, such as the highly endemic Chilean carabid fauna. The level of endemism in Chilean carabids is high (55%) and they are distinctive from the carabid fauna of the rest of South America (Roig-Juñent and Domínguez, 2001). Reducing the use of broad-spectrum insecticides can be a particularly effective mean to conserve generalist predators (Koss et al., 2005). Conservation biological control may rarely be successful as a stand-alone tactic, but rather, needs to be combined with other pest management strategies (Koss et al., 2005). The use of entomopathogenic fungus such as *Beauveria bassiana* rises as a good potential alternative to design an IPM program for pastures considering *D. pallens* as a primary pest and promoting actions that avoid the outbreaks of other herbivores. Some negative effects of *Beauveria* spp. on non-target species have been reported (Lynch and Thomas, 2000) and it is desirable to compare the risk posed by this fungus with the risks of the insecticides currently used against *D. pallens*, before to advocate scaling up its use.

This study was conducted to accomplish two objectives: to compare the effects on generalist predators of both a new biopesticide based on *B. bassiana* and a standard chemical insecticide; and to increase the knowledge of the generalist predator assemblage in Southern Chilean pastures.

Materials and methods.

The study was conducted from October to December 2003 at the experimental field of the Universidad Austral de Chile, Valdivia, Chile. The naturalized pasture was comprised primarily of ryegrass (*Lolium perenne* L.) and Yorkshire fog (*Holcus lanatus* L.), with broad-leaf weeds covering less than 10% of the surface. The ca. 4 ha field was surrounded by a road (one side), pastures (two sides) and a riparian area (one size). Climate is typical of temperate zones and data are shown in Figure 1.

Three treatments were arranged in a completely randomized design, with four replicates (30 x 30 m unfenced plots). One treatment corresponded to the standard chemical insecticide used against *Dalaca* spp., the synthetic pyrethroid lambda-cyhalothrin (7,5 active ingredient per ha, Zero, ANASAC, Santiago, Chile). The second treatment was a new biopesticide developed by the Instituto de Investigaciones Agropecuarias (INIA), consisting of dried spores of the fungus *Beauveria bassiana* coded B-931. Based on previous studies,

a dose of 10^{12} spores per ha was selected. Finally, no treated plots remained as control. All treatments were applied on 15 October 2003, spraying an equivalent to 200 L of tank mix per ha using a standard horizontal bar mounted on a tractor. Control plots were sprayed with water.

Predator sampling.

Surface predators were sampled by dry pitfall traps. Pitfall trap capture of individuals of the same taxa at different locations of the same habitat during the same time period can be compared as a measure of the relative abundance at each location (Maloney, 2002). Plastic cups (370 mL, 10 cm high, 5 cm diameter) drilled at bottom for drainage were used as traps. Sampling was performed once before spraying and three times after spraying (Fig. 1). In each sampling date, traps were active for 4 days and they were checked daily. We put twelve traps per plot, arranging them on 3 rows by 4 columns. The traps were put in the center of each plot and wide mesh plastic net supported by wooden sticks was used to avoid bird foraging on insects captured. Trap catches were pooled over each plot.

All collected arthropods were placed into a 70% ethanol solution and stored until the samples could be sorted under a dissecting microscope. Arthropods were identified comparing them to a reference collection held at Entomology Laboratory, Universidad Austral de Chile and with the help of specialists (see Acknowledgements).

Statistical analysis.

We use one-way analysis of variance to test for differences in relative abundance for each of the five predominant carabid species and two spider families as well as for all seven combined (see Table 1). No statistical analyses were performed on the rare species, although they were included in total trap analysis. When significant treatment effects were detected, means were compared by Fisher's least significant difference test ($p=0.05$). Data were transformed ($\log(x+1)$) before analysis, which were performed in S-Plus software. Analyses were done separately in each sampling date.

Results

A total of 1608 individuals were captured in the sampling period. Ground predators were comprised by carabid beetles (11 species, 71%) and spiders (two families, 29%). The carabid assemblage was dominated by four species accounting by 89% of the captures:

Ferionomorpha nebroides (Curtis) (45%); *Allendia chilensis* (Dejean) (20%); *Argutoridius chilensis* (Dejean) (13%); and *Ferionomorpha aerea* (Dejean) (11%). Seven species accounted for an additional 11% of the captures: *Ceroglossus chilensis* Eschscholtz (6%); *Trirammatus unistriatus* (Dejean) (3%); *Trechisibus angularis* Jeanel (1%); *Calosoma vagans* (Dejean) (<1%); *Mimodromites cyaneus* (Dejean) (<1%); *Metius flavipes* (Dejean) (<1%); and *Parhypates* sp. (<1%). The represented tribes (Table 1) were Pterostichini (72%), Harpalini (20%), Carabini (7%) and Trechini (1%).

The sampling period covered most of the activity of spring breeders such as *F. nebroides*, *F. aerea*, *All. chilensis*, *C. viridis* and *M. cyaneus*. The last two species were not represented enough to test for differences, while *All. chilensis*, which had been abundantly collected before spraying, remained at very low population levels for the rest of the experiment (Figure 2). Before the application of treatments, numbers of ground predators were relatively low, but they were evenly distributed in the plots assigned to each treatment, as no statistically significant differences ($p>0.05$) were detected in the pre-treatment sampling date for any taxa. No species showed an even distribution along the sampling period. The most numerically represented carabid species (*F. nebroides, Ar. chilensis*, *C. chilensis*, *F. aerea*) tended to increase in the latter part of the sampling period, while one species (*All. chilensis*) showed the opposite pattern.

The families Gnaphosidae and Lycosidae were almost equally represented with 54% and 46%, respectively. Both families were relatively scarce at the early part of the sampling period and they tended to increase in the last two sampling dates (Figure 2).

The ANOVA performed on total number of predators at each sampling date indicated that the predators as a whole were decreased by the insecticide in all three post-treatment sampling dates. The decreases were 40, 59 and 51% for 1, 30 and 60 days after spray, respectively (Figure 2). Numbers of total predators in control and *B. bassiana* plots did not differ at any sampling date ($p>0.05$).

At the individual taxa level, only two carabid species (*F. aerea* and *F. nebroides*) and the two spider families were decreased, at least in one sampling date, by lambda-cyhalothrin application. No taxa were affected by *B. bassiana* spores (Figure 2).

Numbers of *F. aerea* increased in time in all treatments (Figure 2), but the slope of the increasing in the control plots was steeper compared to insecticide plots. Numbers of *F.*

aerea were very low at 1 and 30 days after spray, although the differences with control curve were not statistically significant ($p=0.09$ and $p=0.27$, respectively). In the last sampling date, the *F. aerea* activity-density in the control plots was 3,4-fold the activity density in the insecticide plots ($p<0.05$). The *F. aerea* numbers in the *B. bassiana* plots did not differ from those at the control plots ($p>0.05$).

The effect of treatments on *F. nebroides* numbers showed a consistent pattern in the three post-treatment sampling dates: activity density in *B. bassiana* plots was wery similar to control plot, while activity density in lambda-cyhalothrin plots was lower than both of them (Figure 2). However, the differences between insecticide treatment and control were statistically significant only in 1 and 60 days after spray ($p<0.05$). One day after spray, the activity density of *F. nebroides* was decreased by 89% on lambda-cyhalothrin plots, while it was decreased by 62% on 60 days after spray. *B. bassiana* spores did not affected *F. nebroides* activity density at any sampling date.

The two spiders families showed the same response pattern. Numbers of spiders were low in the early part of the experiment, but they begun to increase by day 30 after spraying. However, the increase was much less dramatic in lambda-cyhalothrin plots. Activity density of Lycosidae and Gnaphosidae treated with insecticide represented only the 22 and 31% of the activity in the control plots, respectively ($p<0.05$). No significant effects of lambda-cyhalothrin were detected 1 or 60 days after spraying. *B. bassiana* activity density did not differ from control at any sampling date ($p>0.05$).

Discussion.

Ground predator assemblage composition.

Surface predators were comprised mostly by carabid beetles and spiders, a common result when pitfall trapping is the preferred sampling method. Rove beetles (Staphylinidae) were occasionally trapped as well, but they were excluded from analysis because of low numbers. The carabid assemblage revealed in this study was dominated by mid-sized species, ranged from 6.5 mm (*Ar. chilensis*) to 14.7 mm (*F. aerea*). Smaller species were rare, while large species were present only very late in the sampling period. In particular, one species (*F. nebroides*) did make up 45% of beetles captured.

Species composition was different from what was reported by Zelada (1998) in an early study conducted in the same research field in the same period (October to December). In

that study, the assemblage was comprised by *F. aerea* (46%), *Ar. chilensis* (15%), *C. viridis* (12%), *M. cyaneus* (7%) and *C. vagans* (5%). In our study, the numerically dominant species were *F. nebroides* (45%), *All. chilensis* (20%), *Ar. chilensis* (13%), *F. aerea* (11%) and *C. chilensis* (6%).

Spiders belonging to Lycosidae and Gnaphosidae were represented in the captures, which are active wanderers and expected to be (over)represented in pitfall catches (Young and Edwards, 1990). Lycosid spiders are dominant in many agroecosystems, including Hungarian arable fields (Samu and Szinetár, 2000), soybean and mungbean in Australia (Pearce et al., 2004), blueberry in USA (Maloney, 2002) and many others (Marshall et al., 2002). Many lycosid species have been named agrobiont (*sensu* Luczak, 1979) based on their abundance and synchronization with crops. In contrast, the Gnaphosidae often represented low proportions of total catches compared with lycosids and other families in surveys conducted in other agroecosystems (Samu and Szinetár, 2002; Pearce et al., 2004; Maloney, 2002), while that in our study, Gnaphosidae was as well represented as Lycosidae, from a numerical point of view, differing from the studies cited above. Nevertheless, if biomass is considered, lycosid spiders captured in our pitfall traps represented a higher proportion because they are several times heavier than gnaphosid spiders.

Response to treatments.

Carabidae.

Previous research have shown contrasting responses of ground predators to different pest management programs (O'Neal et al., 2005). In general, when synthetic pyrethroids like lambda-cyhalothrin are sprayed, in many cases the ground beetles are decreased, but usually they recover into 2-3 weeks (Prasifka et al., 2005; White et al., 1990). Indeed, some increases after lamba-cyhalothrin spray have been reported (Volkmar et al., 1996). In our study, negative effects of lambda-cyhalothrin in activity density of predators were detected up to 60 days after spraying.

In carabid species, acute toxicity of lambda-cyhalothrin was not evident immediately after spraying, except for *F. nebroides*. This result must be interpreted with caution, because the catches of *Ar. chilensis, C. chilensis, F. aerea* and *All. chilensis* were very low at mid October. Therefore, the absence of active populations of beetles could be a better

explanation than no toxicity of the insecticide, considering significant effects of lambda-cyhalothrin were detected twice in the following sampling dates and that lambda-cyhalothrin toxicity to carabids is well documented in literature (Huusela-Veistola, 1996; Koss et al., 2005; Prasifka et al., 2005; White et al., 1990), though the response is always species-specific.

F. nebroides showed a consistent response across the study. Activity density in the lambda-cyhalothrin plots was lower than control in the three post sampling dates, although the differences were significant at $\alpha = 0.05$ only at the beginning and at the end of the sampling period, while the response at the intermediate sampling date was not statistically significant ($p=0.29$). Other carabids with comparable body size to *F. nebroides* can move several meters per day (Thacker and Dixon, 1996), but insecticide plots did not recover the pre-treatment populations levels at any sampling date.

The negative effect of lambda-cyhalothrin on *F. aerea* population was evident two months after spray, but not one month after spraying. Absolute numbers increased with time in all treatments, but the increase was much more marked in the control plots than in insecticide plots, in the last sampling date. This species overwinters as adult, thus the increase should be attributed to adults moving from no treated areas to plots and not to new cohorts of adults from larvae that complete their cycle.

The absence of barriers, the presence of migrant sources and the plot size would have facilitated (or at least no make more difficult) the re-colonization of treated plots by *F. nebroides* and *F. aerea*. The reasons why the re-colonization was not accomplished as expected are not clear. If the insecticide had persisted in the treated plots, direct mortality or repellence could be acted on beetles, but lambda-cyhalothrin has been reported to persist less than 2 weeks in field (Hill and Inaba, 1991; Mathirajan et al., 2000). In addition, lambda-cyhalothrin decreases when temperature increases (Huusela-Veistola, 1996) and temperature at November-December in the region (late spring and early summer) are typically higher than October (mid spring).

Several authors have reported that carabids do control lepidopteran pests (French et al. 2004; Frank and Shrewsbury, 2004; Toft and Bilde, 2002). The carabid species active at the sampling period likely would not be predators of *Dalaca* sp. because of their body size. It is generally noted a positive correlation between the size of the beetles and the size of the

prey attacked (Larochelle, 1990; Smith et al., 2004). In mid spring and early summer, *Dalaca* sp. larvae reach 5-6 cm long and 580 ± 167 miligrams (Devotto, unpub. data). This body size is 3 to 4-fold the body size of the most abundant species detected in our study. In our dry pitfall traps, occasionally some *Dalaca* sp. larvae were caught. Carabid beetles readily preyed on them, but only when their numbers were much higher than *Dalaca* sp. larvae. At field, it is likely that the very active and strong *Dalaca* sp. larvae could escape from predation. If some species of the carabid assemblage do prey on mature larvae, potential candidates must be the large carabids such as *Calosoma vagans* and *Ceroglossus chilensis*, species that emerge latter in the season. The first species belongs to a gender commonly called "caterpillar hunters" because *Calosoma* tend to prey on lepidopteran larvae (French et al. 2004;, Toft and Bilde, 2002). Unfortunately, prey range of these species remains unknown, especially on *C. vagans*, which has a pre-oral digestión system.

On the other hand, eggs and neonate larvae are abundant resources for several months in the pastures. The *Dalaca* sp. females bear up to 2000 eggs and they drop them over the pastures in a no directed way (Cisternas, personal comm.). In the *Dalaca* sp. breeding period, hundreds of eggs can be found at random on weed leaves, in the grass and at the soil surface, where they are exposed to predation, as well as neonate larvae. The potential role of carabids and spiders as predators of eggs and neonate larvae must not be dismissed and warrants further research.

Spiders

The lack of high numbers did not allow detecting differences between treatments immediately after spraying. By day 30, lycosid and gnaphosid spiders increased in all plots, but the increase was higher in control and *B. bassiana* plots. The spider numbers kept growing by day 60 in the lambda-cyhalothrin plots, but in the control and *B. bassiana* plots the spider numbers were similar (lycosids) and lower (gnaphosids) than the spider numbers in the same plots in the previous sampling date. We did not distinguish spiders by sex nor did we record the size of the spiders trapped, but the authors noted females bearing egg sacs and spiderlings only late in the sampling period, therefore the increase in insecticide plots by day 60 could be a re-distribution of adults rather than recruitment of new individuals. The size plot must not be an obstacle to cursorial movement of spiders, though they can cover considerable daily distances (Kiss and Samu, 2000). These findings confirmed that

fields that are sprayed with pesticides such as lambda-cyhalothrin often have lower spider populations (Wehling and Heimbach, 1991; Maloney, 2002), as a consequence of the high spider susceptibility to this kind of insecticide (Krause et al., 1993).

Lycosid spiders prey on lepidopteran pests in several crops: corn (Laub and Luna, 1992), rice (Wilby et al., 2005), soybean (Pearce et al., 2004), apple orchards (Allen and Hagley, 1989). Although the diet of spiders in the Chilean pastures is unknown, top-down effects of spiders are evident even when they do not actually feed upon the target pest (Greenstone, 1999). Often, insect herbivores reduce their feeding when in the presence of spiders, and disperse or abandon high quality patches (Sunderland, 1999). It has been stressed that an assemblage of spider species is more effective at reducing prey densities than a single species of spider (Greenstone, 1999; Sunderland, 1999). As suggested by Greenstone (1999), it is important to have an assemblage of spiders rather than just one species so predators of appropriate size classes and foraging modes will be present to prey upon different prey life stages throughout the growing season. Therefore, it would be desirable for growers to have a more selective tool than lambda-cyhalothrin if conservation of spider assemblage is attempted.

Indeed, it seems that spider species abundant in pastures (agrobionts and agrophiles) are not the same present in surroundings (Martin and Major, 2001; Alderweireldt, 1989). Therefore, edges would not be primary migrant sources for the agrobionts and agrophiles spider species, making more difficult for local populations recovering from depressed levels caused by insecticide spraying. Lycosid spiders migrate basically by cursorial movement (Bishop and Riechert, 1990). Ballooning, the colonization mean over long distances, is possible just for juveniles less than 5 mm (Pearce et al., 2004; Bell et al., 2001), then the capacity of spiders to recolonize areas where they were removed is limited over long distances. Therefore, to avoid spider local extinctions in pastures is highly desirable and this can be accomplished if, for instance, lambda-cyhalothrin spraying is restricted to crucial periods in the pest life cycle, spraying at midday when many wandering spiders are inactive and in sheltered location (Riechert and Lockley, 1984) or preferring other less disruptive tools such as the *B. bassiana* spores tested in this study.

In small-scale within-field trials, the impact of pesticides may be underestimated (Duffield and Aesbicher, 1994; Prasifka et al., 2005), because individuals can migrate from the

within-field control plots as well as surrounding untreated fields (Thacker and Dixon, 1996; Huusela-Veistola, 1996; Duffield and Aebischer, 1994). Indeed, Jepson and Thacker (1990) demonstrated a significant positive correlation between the scale on which the experiment was carried out and the duration of the treatment effect. We raise concerns on spraying of broad spectrum insecticides such as lambda-cyhalothrin to control *Dalaca* sp. based in our findings because persistent negative effects were detected despite of small plot size and absence of barriers of movement. In addition, the 4 most abundant species are spring breeders and if egg-bearing females are removed from population, negative consequences for that local population could extent to the next generation. The predation on items used by adult in the current season and by the larvae in the summer and autumn could be relaxed, increasing the chance of herbivores outbreaks. This situation would be stressed if we consider that in practice, whole fields are treated and no control plots exist, therefore less migrant sources are available.

While single predator taxa can, at times, have a strong impact on checking a target pest, there is growing evidence that diverse guilds may be more effective (Koss et al., 2005). This has been suggested for carabids (Symondson et al., 2002) as well as spiders (Greenstone, 1999; Sunderland, 1999). Therefore, we advocated for the conservation of the whole surface predator assemblage rather than a single species.

Conclusions.

The spring surface predator assemblage was make up mostly by carabids belonging to *Ferionomorpha nebroides* (Curtis), *Allendia chilensis* (Dejean), *Argutoridius chilensis* (Dejean) and *Ferionomorpha aerea* (Dejean). The spiders were represented equally by two families, Lycosidae and Gnaphosidae, differing from spider assemblages surveyed in other countries and agroecosystems.

No species was affected by *B. bassiana* at the applied dose, despite of significant numbers of spores were present in soil (up to 15 days) and foliage (up to 7 days) and that *B. bassiana* isolate B-931 was able to kill at least one carabid species in laboratory (Devotto, unpub. data). This finding confirmed results from other field studies on mass-release of *B. bassiana,* which reported than negative effects on non target species are no existent or minimal (Lynch and Thomas, 2000). In contrast, the insecticide lambda-cyhalothrin affected at least two carabid species and the two spider families. The recovery times of

affected species, when present, where longer than those often reported in literature. Therefore, the use of this fungus would pose less risk to the generalist predators of Southern Chilean pastures than the synthetic pyrethroid used for *D. pallens* suppression.

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Table 1. Composition of spring surface predators in a Southern Chilean pasture (October to December, 2003), revealed by pitfall trap sampling.

Taxa	Number of individuals captured	Percentage
HARPALINI		
Allendia chilensis	223	19.6 %
Mimodromites cyaneus	4	0.4 %
PTEROSTICHINI		
Argutoridius chilensis	149	13.1 %
Feroniomorpha aerea	120	10.6 %
Feroniomorpha nebroides	509	44.8 %
Metius flavipes	1	0.1 %
Parhypates sp.	4	0.4 %
Trirammatus unistriatus	33	2.9 %
CARABINI		
Calosoma vagans	8	0.7 %
Ceroglossus chilensis	73	6.4 %
TRECHINI		
Trechisibus angularis	12	1.1 %
TOTAL GROUND BEETLES	1136	71 %

ARANEAE		
Gnaphosidae	253	54 %
Lycosidae	219	46 %
TOTAL SPIDERS	472	29 %
TOTAL PREDATORS	1604	100 %

Figure 1. Environmental data for the sampling period (air mean temperature, top 1 cm soil mean temperature and rain). Pesticide spraying is indicating by black vertical arrow. Active periods of pitfall trap sampling are indicating by horizontal arrows.

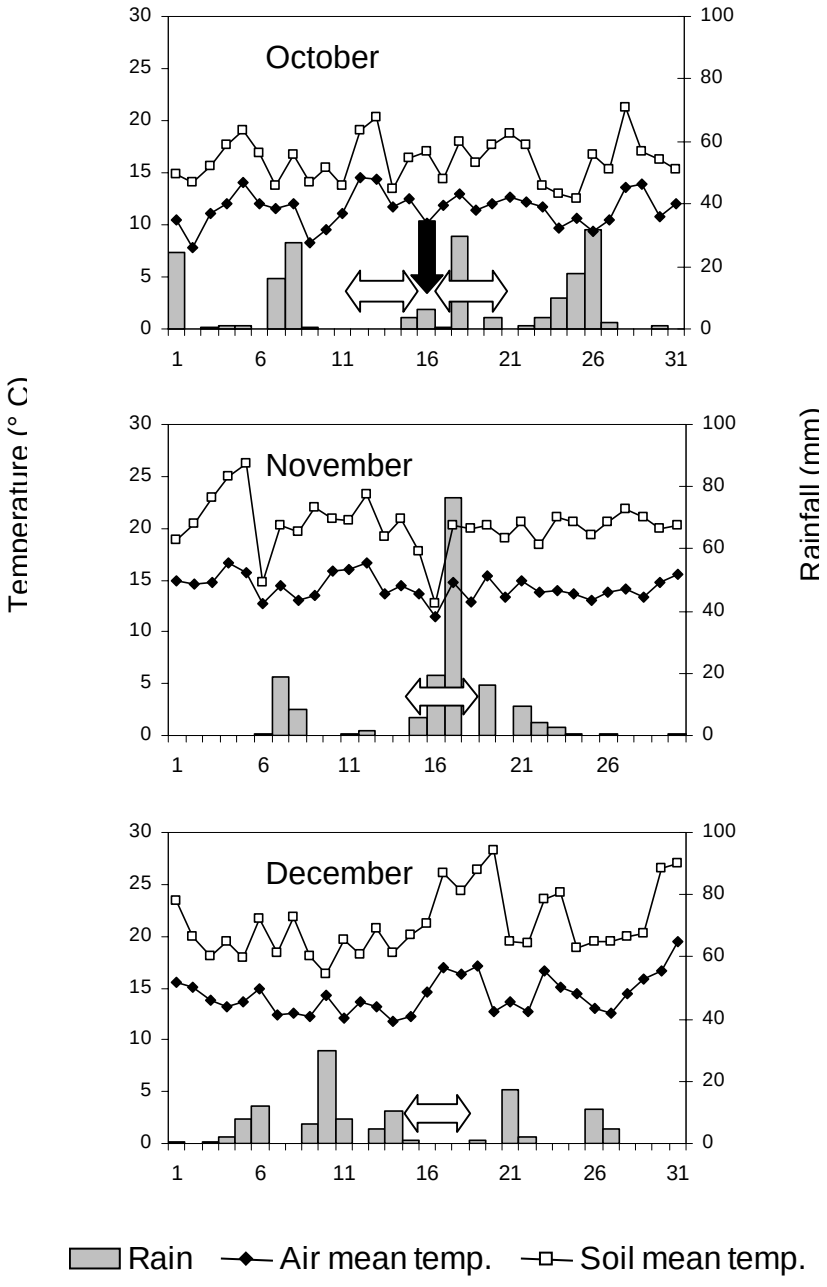
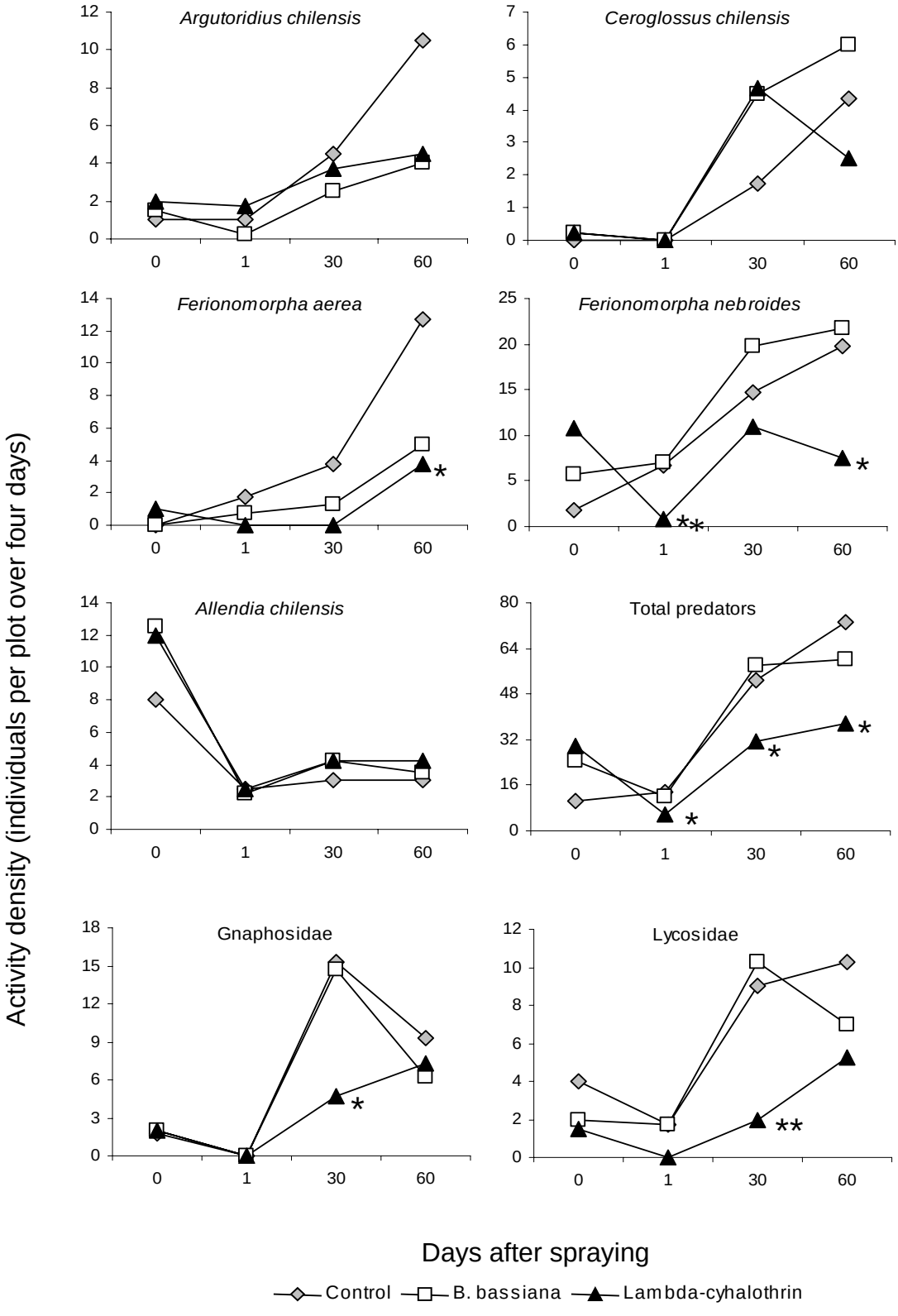


Figure 2. Activity density of selected taxa before and 1, 30 and 60 days after spraying of *Beauveria bassiana* spores or lambda-cyhalothrin. Treatment means differing from control mean, according to Fisher's LSD, are indicating by * ($p<0.05$), ** ($p<0.01$).



3.- CAPÍTULO TERCERO: ANÁLISIS DE LOS EFECTOS NO DESEADOS A NIVEL DE GREMIOS.

Este artículo puede ser consultado bajo el título:

Devotto L., R. Carrillo, E. Cisternas and M. Gerding. Non-target effects of *Dalaca pallens* control in South Chile: an analysis of biological and chemical control at the guild level. Agriculture, Ecosystems and Environment (submitted).

NON-TARGET EFFECTS OF *Dalaca pallens* CONTROL IN SOUTH CHILE: AN ANALYSIS OF BIOLOGICAL AND CHEMICAL CONTROL AT THE GUILD LEVEL.

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Summary.

The larval feeding activity of *Dalaca pallens* (Lepidoptera: Hepialidae) reduces severely the pasture yield. A field experiment was conducted to compare the effects of *Beauveria bassiana* (10^{12} spores/ha) and the insecticide lambda-cyhalothrin (7.5 g active ingredient/ha) on non-target predator, herbivore and decomposer guilds. Principal response curves (PRC) were performed to test the treatment effects over time and standard ANOVA was used to confirm the PRC results on some individual taxa. Soil cores and pitfall trap sampling were used to measure the abundance or activity of taxa before and several dates after spraying. Using an oat-dodine selective medium, spores in soil increased by 70% after application. Spore numbers dropped to pre-treatment levels one and two weeks in foliage and soil, respectively. The predator guild was severely disturbed by the insecticide, although no adverse effects of lambda-cyhalothrin was observed on herbivore or decomposer guilds. No guild or individual taxon was affected by *B. bassiana* spores. The negative effects of insecticide were present from 1 to 60 days after treatment. Several carabid species and lycosid spider populations were depressed the most. Potential consequences for natural pest control in Chilean Southern pastures are discussed.

Keywords: Pasture pest; Principal curve response; Entomopathogen; Non-target effects; Guilds.

Introduction.

Three millions hectares are dedicated in Southern Chile to support beef and dairy cattle, the most important agricultural activity in that area of the country. Yearly, about 10% of this area is sprayed with synthetic insecticides to control the pest *Dalaca pallens* (Blanchard) (Lepidoptera: Hepialidae). The larval feeding activity of this moth reduces severely the pasture yield and high infestations can produce the death of the plants. Growers keep this pest in check mainly by massive spraying of pyrethroids and insect growth regulators (IGRs). However, more alternatives are needed to design a sound and robust control scheme under the integrated pest management approach.

Beneficial arthropods play an important role in controlling crop pests and thus the selectivity is a fundamental component of IPM in order to minimize disruption of the ecological community, especially natural enemies. The application of broad-spectrum insecticides often disturbs the activity or abundance of natural enemies and thus depresses the natural control of pests. In addition, species other than predators or parasitoids are being recognized as useful and necessary for a proper functioning of agroecosystems (Kenmore et al., 1984; Settle et al., 1996). Both toxicological and selective properties of a material must be evaluated before to include a new product in the IPM of a pest.

The fungus *Beauveria bassiana* (Balsamo) Vuillemin is a world-wide entomopathogenic organism and is used to control several insect pests in many countries. The Entomopathogenic Organisms Collection held at the Instituto de Investigaciones Agropecuarias (INIA) includes more than three hundred *B. bassiana* isolates collected across the country. The isolate B-931, originally collected from a field-parasitized larva of *D. pallens*, has been under research for more than 5 years to assess its efficacy against *D. pallens* in laboratory, small field and large field experiments (Cisternas, unpubl.[*]). A field, large plots (1 ha) and replicated experiment carried out in 2003 (Cisternas, unpubl.*) showed that the application of ten grams of spores per ha (10^{12} spores per ha) caused the

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same percentage of mortality on larva than the insecticide lambda-cyhalothrin (>80% of efficacy).

Like any pest control technology, *B. bassiana* may present a risk to non-target species, including the natural enemy community. Laboratory tests have indicated some level of acute adverse effects on a suite of individual non-target organisms (Traugott et al., 2000; Danfa and van der Valk, 1999), while field studies have reported minor or non existent deleterious effects of *Beauveria* sp. spores on non-target species (Riedel and Steenberg, 1998; Lynch and Thomas, 2000).

On the other hand, few laboratory and field studies have addressed the non-target effects of insecticides currently in use against *D. pallens* and less studies have been conducted at the supra-species level. We choose two sampling methods (soil cores and pitfall sampling) and a relatively new tool based on ordination called principal response curve (PRC), to evaluate the predator, herbivore and decomposer guild responses to *D. pallens* control. Analysis of PRC was especially designed for mesocosms experiments (van den Brink and ter Braak, 1999) and has been extended to other fields in recent years (Naranjo, 2005; Naranjo and Akey, 2005; Naranjo et al., 2004; Candolfi et al., 2004; Dively and Rose, 2002).

We used this approach because the experiments on non-target effects produce large data sets on which standard univariate statistical methods do not perform well in all cases, mainly because the data often are over-dispersed and a large number of sequential zeroes are present. Multivariate analysis may be used to describe the effect of chemical stress at the assemblage level (van den Brink and ter Braak, 1999) and the PRC displays, in a single graph, the treatment effects over time and allows to determinate the statistical significance of the effects by Monte Carlo (MC) permutation testing (van den Brink and ter Braak, 1998).

The aim of this study was to compare the short-term effects of biological and chemical control of *D. pallens* on several guilds (predators, herbivores and decomposers). For this purpose, we carried out an experiment which we assessed the activity/abundance of these guilds before and after a single application of the *B. bassiana* isolate B-931 and the synthetic insecticide lambda-cyhalothrin.

Materials and Methods.

Site.

The experiment was carried out at the experimental field of Universidad Austral de Chile, Valdivia, Chile (39°47'04" S, 73°13'45" W), from October to December 2003 (growing season in Southern spring). The soil type is characterized as medial mesic typic hapludand, pH 5.8 and 15% of organic matter. Standard agronomic practices were used including fertilization with NPK (54, 110 and 48 units respectively), but no herbicides or other plant protection products were used except for the products tested in the study (see below).

Fungus.

A *B. bassiana*-parasitized larva of *D. pallens* was found in a field near to Osorno, Chile, in 1998. The isolate was coded B-931 and is held at –196 °C for long-term storage in the Entomopathogenic Organisms Collection, Instituto de Investigaciones Agropecuarias (INIA) Quilamapu, Chillán, Chile. Sub-samples of the original sample have been extracted in time to mass-rear this isolate on rice bugs, according to a method modified and adapted to INIA conditions (France, pers. comm.[*]). Spore dose was measured by microscope using a counting slide. Viability of spores were checked on agar plates for 48 h. Over 80% of spores were viable.

Treatments.

1. Control. The control plots were sprayed with water (equivalent to 200 L per hectare) and 10 mL of soft detergent (Down, Procter&Gamble, Bs. Aires, Argentina).

2. *B. bassiana*. Spores of the isolate B-931 were sprayed once at a rate of 10^{12} spores/ha. Dried spores were mixed with a soft detergent (Down, Procter&Gamble, Bs. Aires, Argentina) and then the mix was added to the tank. The tank mix was sprayed with a standard horizontal bar mounted in a tractor (6 m wide).

3. Lambda-cyhalothrin. This insecticide was included in the study as a positive control. The commercial product Zero 5 EC (ANASAC, Santiago, Chile), and water were mixed in the tank as described above and applied at label rate (7.5 g of active ingredient/ha, 150 cc of formulation/ha), using the same procedure of spraying that in treatment 2.

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Treatments were applied on 15 October 2003, after 18:00 h, with weather conditions of 12.5 (air) and 16.4 (soil) °C, cloudy day with weak showers (rainfall is showed in Figure 1). The pasture was 15-20 cm high. All applications were made by tractor-mounted sprayer at 200 L per ha.

Data collection.

Pitfall trapping. Pitfall traps are widely used for ground-dwelling arthropod sampling (Spence and Niemelä, 1994). Because many factors aside from abundance influence pitfall trap captures, we adopted the term activity-density following Thiele (1977) to record and show our results. Pitfall traps were made from one 370 mL blank plastic cup (10 cm high, 5 cm diameter), with no cover. The cups had very small holes drilled in the bottom for drainage. Twelve traps per plot were buried in the soil so that the lip was flush with the soil surface and arranged on a grid, on 3 columns by 4 rows. The traps were put in the center of the plot and the grid area was covered by a net to prevent bird foraging on caught arthropods. The unbaited traps were 0.5 m each other and were active for a 4-days period in each sampling date. When the traps were not active, they were inverted. The arthropods were removed from traps every morning. Sampling was performed four times during the study: before spraying and 1, 30 and 60 days after spraying. Activity-density from pitfall trap captures was shown as the average number of individuals per trap in each 4-d sampling period.

Soil cores. Thirty soil cores (9 cm diameter, 10 cm high) were extracted at random in each plot, although they were not extracted close to the plot borders to avoid potential edge effects. The cores were bagged to prevent arthropod escape or loss. Invertebrate were extracted by Berlese-Tullgren method for 96 h. The cores were put inverted in extractor over a layer of cheese-cloth over screen of extractor to prevent soil particles being dislodged into the extractant. A mix of water and a soft detergent were used as extractant. The content was sifted over a 2 mm mesh screen and then sifted again over a finer mesh screen. Large specimens were separated by hand or with forks, while the remaining content was rinsed with tap water over a <1-mm mesh screen and specimens were removed with a fine paintbrush. Samples were placed on 70% ethanol. Figures from soil cores were transformed to number of individuals per squared meter.

Spore persistence in soil. Six-eight soil cores (9 cm diameter, 10 cm high) were extracted at random in each plot. They were pooled on a plastic bag to get ca. 1-1.5 k of soil and cooled (5-10° C) prior to analysis. Spores number was estimated through dilution plating method described in detail by Chase et al., 1986. We used dodine fomulated as Syllit 65 WP (ANASAC, Santiago, Chile). The procedure was as follows:

In the laboratory, the soil was sieved and litter and plant parts were removed. Fifteen grams of fresh soil were added to a flask with 25 mL of sterilized distilled water and drops of Tween-20 as surfactant. In parallel, three samples of 100 g of soil were dried in a stove to measure the soil water content. The mix was shaken by hand for 5 min. An aliquot (2.5 mL) was transferred to a second tube and then sterilized water and Tween-20 were added to complete 25 mL. The new tube was treated as above and when all the dilutions were available (10^{-1} to 10^{-3}), we transferred 150 µL of suspension, using a pipette, to each plate with selective media (3 plates per dilution). The plates were cultivated for ten days (no light, 20° C) and colony forming units (CFU) were recorded at the end of this period. A proportion of the colonies was sampled and correct identification was confirmed by microscopic exam. Counts were corrected by water content to express the spore numbers on a dry soil basis. Spore number in soil was estimated five times in the study: before and 1, 5, 15 and 66 days after spraying.

Persistence of spores on leaves. Foliage samples were collected at random in each plot (10-15 points) and pooled. In the laboratory, pieces of foliage were cut with scissors and measured to complete 32 cm^2. Leaf pieces were added to a tube with 25 mL of sterilized distilled water and drops of Tween-20. Then, the same procedure of soil samples was adopted. Spore numbers are expressed by fresh leaf area. Just ryegrass leaves were included in this analysis. Sampling was performed at 1, 4 and 7 days after spraying.

The dilution/transference process was repeated 4 times, therefore dilutions from 10^{-1} to 10^{-3} were available, both soil and foliage samples. Estimates from dilution 10^{-2} were used to draw Figure 1.

Arthropod identification.

Specimens were stored on 70% ethanol. Samples were sorted in the laboratory with the aid of a microscope and identification was performed with reference to Artigas (1994), CSIRO (1991) and with the help of a reference collection identified by Dr. Roberto Carrillo

(Departamento de Producción y Sanidad Vegetal, Universidad Austral de Chile) over the last ten years. Some specimens were not identified to the species level, thus the terms "taxa" and "species" are used interchangeable in this paper. Voucher specimens were hold in the Quilamapu Regional Research Centre, INIA, and the Entomology Laboratory, Universidad Austral de Chile.

Data sets.

Four data sets were available:

Predator guild from pitfall trapping (data set I). This data set comprises two spider families (Lycosidae and Gnaphosidae) and 11 species of carabid beetles: *Mimodromites cyanaeus* (Dejean), *Trechisibus angularis* Jeanel, *Calosoma vagans* (Dejean), *Metius flavipes* (Dejean), *Parhypates* sp., *Trirammatus unistriatus* (Dejean,), *Allendia chilensis* (Dejean), *Argutoridius chilensis* (Dejean), *Ceroglossus chilensis* Eschscholtz, *Ferionomorpha aerea* (Dejean) and *Ferionomorpha nebroides* (Curtis).

Predator guild from core sampling (data set II). This data set comprises six taxa: the spider families Gnaphosidae and Lycosidae, the rove beetle family Staphylinidae, the earwig *Forficula* sp., and the taxa Carabidae (larva) and Carabidae (adult). The carabid adults were pooled because they were present at low number. The Carabidae (larva) was treated as separated taxa because the larva were not unequivocally assigned to an species.

Non-target herbivore guild (data set III). The taxa included in this data set were extracted from soil cores by the Berlese method. The eight taxa were: the ryegrass weevil *Listronotus bonariensis*, the diptera Tipulidae, the families Elateridae, Noctuidae, Cantharidae, Curculionidae (larva), the weevil *Apion* sp. and the taxa Other Coleoptera.

Decomposers guild (data set IV). These specimens were extracted from soil cores as above and comprimes four taxa: earthworms (Lombricidae), larva of the Stratiomyiidae family, the Oribatidae mites and Coleoptera.

Statistics and analysis of data.

The experiment was arranged in a completely randomized design, with four replicates. The size of each plot was 30 x 30 m. Multi-variate analysis were conducted to test whether the treatment regimes affected the activity or density of selected guilds.

Multivariate statistics. Principal response curves (PRC) were used to analyze the time and treatment-dependent multivariate response of non-target assemblages. Background of this multivariate analysis is given by van den Brink and ter Braak (1999). In brief, this method summarizes all the information of the recorded assemblage simultaneously. The principal response, which is a weighted sum of the abundances of the taxa, was expressed as a canonical coefficient and reflected the behavior of the treated assemblages relative to the untreated control (Dively and Rose, 2002). In addition, PRC makes assemblage-level responses easier to plot and interpret and can deal with the large number of zeros often found in ecological community data.

Monte-Carlo permutation tests (1999 permutations) were used to test the significance of the treatment effects (departures from the zero control line) at each sampling date. The partial redundancy analysis and MC testing were performed on CANOCO software version 4.53 (ter Braak and Smilauer, 1998). Input data were log(x+1) transformed for analysis. CANOCO outputs were used to calculate the PRCs in a spreadsheet using the formula:

$$Cdt = (TAU \times Regr{:}AX1) / SD \quad (1)$$

Where Cdt = standardized canonical coefficients; TAU = total standard deviation of the species data; Regre:AX1 = Regression/canonical coefficients for standardized variables; and SD = standard deviations of environmental variables.

Results.

Over 4111 specimens were enumerated and the numerically most important taxa were the carabid beetles (42%), oribatid mites (20%), gnaphosid spiders (16%) and curculionid weevils (8%). The remaining taxa accounted by the 14%. In terms of ecological functionality, 61 % of the invertebrates were predators, 11 % were herbivores and 28 % were decomposers.

Spore persistence.

The spore persistence on foliage and soil is shown in Figure 1. Before treatment, *B. bassiana* spore number was 1,7 x 10^4 CFU/dry soil gram. One day after treatment, *B. bassiana* spores increased by 70% (2,8 x 10^4 CFU/dry soil gram), but numbers dropped to pre-treatment level by day 15. Spores in foliage peaked 82 CFU/cm^2, but the number decreased to almost zero one week after spraying.

Predators.

Data set I. The principal response curves of the predator guild recorded by pitfall trapping are shown in Figure 2. The first PRC was significant and explained the 52% of the variance explained by the treatment regime (Table 1). The second PRC explained an additional 25% of the variance, but it was not significant (p=0.10). The first PRC showed that small variations were present in the pre-treatment period and significant deviations from the control in insecticide plots occurred in the three post-application sampling dates.

More detailed information about the significance of treatment effects was gained by performing the MC permutation tests individually for each sampling date. The pre-treatment predator guild did not differ between treatment and control plots (Table 2). One day after the application of the spores or the insecticide, the predator guild activity decreased in the insecticide plots compared with the control plots (p=0.02), while the response curve for the *B. bassiana* plots fluctuated close to the zero line of the control indicating that no significant (p=0.68) changes in guild activity density occurred. The date by date contrasts indicated that this pattern was present from the day 1 throughout the day 60, thus the predator guild did not recover into the sampling period (Figure 2). Overall predator guild in the insecticide plots was reduced by 49% after application of lambda-cyhalothrin, while the predator density activity was reduced by 19% in the *B. bassiana* plots, compared with the control. However, only the lambda-cyhalothrin decrease was statistically significant ($p<0.05$).

For individual responses we checked the species weights, as they denote the relative contributions to the principal response (strength of the response of each taxon). Taxa with high positive weights follow the same pattern of the principal response, whereas taxa with negative values behave in the opposite way that it is indicated by the PRC (van den Brink and ter Braak, 1998; 1999).

The weighted scores for each taxa were ranked in Figure 2 (right). Main contributors to the negative impact observed in the insecticide treatment were *F. aerea*, *F. nebroides*, Lycosidae and Gnaphosidae.

Data set II. The assemblage extracted from soil cores was very similar to the assemblage revealed by pitfall trapping. The only taxon was present in the soil cores but not at pitfall trapping was the rove beetle family Staphilinidae.

The first PRC was significant ($p<0.01$) and accounted for the 87% of the variance explained by the treatment regime (Table 1). The second PRC explained an additional 11% of the variance, but it was not significant ($p=0.59$). No differences occurred between predator guild in the treated and control plots before treatments. Thirty days after treatment, the predator guild abundance was reduced by the lambda-cyhalothrin treatment (Table 3), on the basis of the MC permutation tests ($p=0.02$), while the predator guild abundance was similar between *B. bassiana* treatment and the control ($p=0.77$). The predators as a whole were reduce by 74% in the insecticide plots compared with the control plots (Figure 3).

The weighted scores for each taxa are shown in Figure 3 (right). Main contributors to the adverse effects of lambda-cyhalothrin were carabid adults, Gnaphosidae spiders and carabid larva.

Herbivore guild (data set III). The herbivore guild measured by soil cores sampling consisted primarily of weevils (78%), including *Apion* sp. (34%), the Argentine ryegrass weevil *L. bonariensis* (25%) and curculionid larvae (20%). In order of abundance, curculionids were followed by Cantharidae (6%) and other Coleoptera (12%), while the remaining taxa accounted by less than 2% of the total individually.

The first PRC captured the 47% of the variance explained by the treatment regime, but it was not significant ($p=0.79$, Table 1). Herbivore guild in the pre-treatment sampling date was similar between treatments (Figure 4). Thirty days after treatment, no significant effects of treatments on the herbivore guild abundance were detected, on the basis of the MC permutation tests (Table 3).

Decomposer guild. (Data set IV). The first PRC captured the 77% of the variance explained by the treatment regime, but it was not significant ($p=0.77$, Table 1). Decomposer guild in the pre-treatment sampling date was similar between treatments (Figure 5). Thirty days after treatment, no significant effects of treatments on the herbivore guild abundance were detected, on the basis of the MC permutation tests.

Discussion

The guilds responded differentially to treatments. While herbivore and decomposer guilds were not affected by treatments, predator guild was decreased by lambda-cyhalothrin in all post-treatment sampling dates. This response was consistent considering that abundance of

predators extracted from soil cores followed the same pattern that predator activity density from pitfall trapping. The soil cores estimation was performed 30 days after spraying, thus it comprised the first two sampling dates of pitfall trapping estimates.

The predator guild was numerically dominated by carabid beetles (68%) and spiders (26%). Much research efforts have been focused in these two groups because a) most of the species are polyphagous predators and thus the taxonomic groups as a whole are considered beneficial (Duelli et al., 1999); and b) they have properties for biodiagnostic purposes and thus have an bioindicative value (Marc et al., 1999).

Negative effects of lambda-cyhalothrin on ground-dwelling predators, especially carabids and lycosid spiders, have been widely reported on literature (Wehling and Heimbach, 1991; Brown et al., 1990; Krause et al., 1993; Hof et al., 1995). Some authors have characterized the effects as weak or transient (Candolfi et al., 2004; White et al., 1990; Wick and Freier, 2000; Berg et al., 1998), while others reported that the effects were strong or persistent (Dinter and Poehling, 1992; Dinter and Poehling, 1995; Rose, 2005). Some groups increased after lambda-cyhalothrin application (Wick and Freier, 2000).

On the other hand, some *B. bassiana* strains have caused mortality on non-target predator at the laboratory level, but in general field effects of this fungus are negligible or non existent (Riedel and Steenberg, 1998; Wang et al., 2001, but see James et al., 1995; Jaronski et al., 1998; Flexner et al., 1986), even when a dose as high as 10^{14} spores per ha was applied in forests (Parker et al., 1997). Limited epizootic of *B. bassiana* on staphylinids were reported by Steenberg et al., 1995.

In our study, lambda-cyhalothrin decreased the predator guild by 49-74%, depending on the collecting method (weighted mean = 54%). This effect was almost constant throughout the experiment, as the magnitude of the decrease was similar in each sampling date. No recovery was observed at the last sampling date.

Insecticide application reduced predator activity-density immediately beginning from 1 day after treatment. This instantaneous effect could be attributed to direct toxicological properties of lambda-cyhalothrin on predators, but the persistent decrease (at least 60 days) could be the result of more complex ecological mechanisms. Acute toxicity of lambda-cyhalothrin could not persist for 60 days in the pasture, especially if high temperatures and organic matter are present. Therefore, mechanisms such as loss of habitat quality, lack of

recruitment or depletion of prey can not be rule out as potential explanations. Following the classification scheme for pesticide effects suggested by Hassan (1992), the lambda-cyhalothrin effects can be considered moderately harmful (51-75% of mortality or reduction on beneficial activity) and persistent (more than 30 days).

The *B. bassiana* isolate B-931 did not caused any significant adverse effects on studied taxa, which agreed with several other studies carried out on different *B. bassiana* strains or isolates. Predators showed a trend to decrease in *B. bassiana* plots ranging from 19-27%, depending on which data set was considered (weighted mean = 22%), but these decreases were not statistically significant in any date. In consequence, following the severity indexes for non-target effects of biological control agents suggested by Lynch and Thomas (2000), our field results ranked 0 or 1 (less than 5% of mortality induced by infection, with no recorded significant population consequences). Therefore, the isolate B-931 posed a risk lower than other *B. bassiana* isolates used at field, such as *B. bassiana* ARSEF 2883 (James et al., 1995) or *B. bassiana* GHA (Jaronski et al., 1998). Spores were alive in significant numbers less than one week on foliage and less than 15 days in soil. Therefore, they degraded very quickly under field conditions and thus non-target species were exposed only during a limited period of their life-span. This short exposure period and low dispersal capability are fundamental to consider *B. bassiana* as a safe biological control agent and could explain, at least partially, the lack of deleterious effects on non-target species. The inherent selective properties of *B. bassiana* could play an important role as an explanatory mechanism for the lack of adverse effects. In general, each strain has a narrow host range and infectivity decreases in heterologous hosts.

The disturbance caused by lambda-cyhalothrin on predators may pose a threat both biodiversity and the proper ecosystem functioning, including natural pest control. The level of endemism in Chilean carabids is high (55%), despite of Chilean carabid fauna represents just the 9% of the South American carabid species (Roig-Juñent and Domínguez, 2001). Repeated insecticide inputs can result in the dominance of a few tolerant species, thereby changing the predator community in the long term (Lee et al., 2001).

Despite of relatively few information is available on ecological structure and functioning of Chilean pastures, in recent years gut contents and exclusion experiments (Carrillo, unpubl.*) have given increasing evidence of the importance of ground-dwelling predators,

particularly carabids. Most of the studied carabids feed on a range of prey, including animal (spiders, aphids, coleopterans, dipterans, Lepidoptera larva and hymenopterans) and not animal materials (pollen and fungi). The gut content analysis showed that 65-80% of content was animal, therefore the species studied (*T. unistriatus, A. chilensis, F. aerea*) were predominantly carnivorous. These studies would corroborate previous findings (Lövei and Sunderland, 1996; Kromp, 1999) on importance of carabids as natural pest control agents. Augmenting generalist predator populations could potentially aid in the establishment of balanced ecosystems that are less susceptible to pest outbreaks (Mathews et al., 2004), while enhancing or retention of an assemblage of generalist predators rather than a single species, has the potential for increasing the biological control of diverse and multi-generation pest complexes (Brown and Adler, 1989).

To our knowledge, lycosid and gnaphosid spiders have been not quantified in Chilean pastures, but radio-nucleotide predation experiments conducted in other grassland systems have shown that spiders consumed a high proportion of the herbivores biomass, even over coleopteran predation (Riechert, 1999 and included references). Most of the spiders have long life cycles and generalist feeding habits, therefore they have limited abilities to exhibit density-dependent tracking of their preys (Riechert, 1999). On the other hand, spiders fit better to a equilibrium point model which can be applied to relative stable systems as perennial pastures. Therefore, the substantial decrease on spider numbers could alter the arthropod community dynamics and it is unclear if other groups could play the same function, considering they exert influence on prey dynamics through ways different from predation such as to cease feeding by the predator presence, to forage at less favorable sites and to drop off host plants altogether in an escape response (Riechert, 1999), with a final slowing of prey population growth.

The lost of predator species could lead to outbreaks of secondary pests, considering that the predator species could be act as a keystone species or that intra-guild predation could relax natural control of some herbivore species present in the pasture.

In our study oribatid mites tended to increase in lambda-cyhalothrin plots. This kind of resurgence after pyrethroid application has been found by other authors (Dively and Rose, 2002). Profusion of decomposer, including mites, has been linked to increases in predator densities (Badejo et al., 1995), as they are potential prey items (Lövei and Sunderland,

1996). Although the link between “bottom-up” prey resources in the habitat and predator abundance has been demonstrated in some systems, its importance to the biological control of herbivores has not been well established (Mathews et al., 2004 and references in). The observed increase in oribatid mites did not correlated positively with their potential carabid predators, at least in the short term. However, in our study the smaller species, which could be potential mite predators, were collected at very low numbers, thus they could not forage on this extra resource at the time of the experiment.

The results, especially for predators, warrants further investigation because the broad-spectrum insecticides will continue playing a role in the *D. pallens* control. Anthropogenic pastures are considered relatively impoverished compared to more natural habitats, but they contribute to the biodiversity of Southern Chilean ecosystems because of their extension and patchy landscape.

Use of microbial pesticides for integrated pest management (IPM) has increased in recent years in part because of the low selectivity of conventional insecticides. Biopesticides based on *B. bassiana* have characteristics readily amenable to accomplish IPM requirements, but concerns for the environment has resulted in greater scrutiny of both old and new plant protection products (Solomon and Giesy, 2001), including *B. bassiana*. Before widespread use of these fungi can be advocated, a comprehensive assessment of their impact on the non-target species are needed (Parker et al., 1997). An assessment of the ecological risks of *B. bassiana* should include a comparison with the risks of conventional control methods, as it was performed in this study. On the other hand, the risk and hazard assessment process is completed just when the different stages (hazard identification, exposure assessment, effects assessment, risk characterization and risk management) are accomplished (Römbke and Moltmann, 1996). Therefore, more detailed knowledge of invertebrate community dynamics on Chilean pastures is needed to enhance pest control and to ensure that this new technology is safer than the current techniques in use.

Finally, results of this study supported the findings reported on early literature, which showed that the use of *B. bassiana* spores at field do not pose a significant hazard for any recorded non-target guild or taxon, while the use of a broad-spectrum insecticide like lambda-cyhalothrin severely disrupted the predator assemblage. The ecological consequences of this disruption can not be anticipated by a short-term study like this, but

the consistent results allow us to rise concerns on natural control of pest and conservation of natural enemies.

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Table 1. Variance allocation of tested data sets. Significance of the PRCs is indicated in brackets (Montecarlo permutation tests, 999 permutations).

Data set	% variance accounted by:		% variance explained by treatment regime captured by:	
	Time	Treatment	First PRC	Second PRC
Predator guild I	42.4 %	18.5 %	51.7 % (p=0.01)	25.2 % (p=0.10)
Predator guild II	33.0 %	28.1 %	86.6 % (p<0.01)	11.1 % (p=0.59)
Herbivore guild	26.8 %	14.1 %	47.1 % (p=0.79)	36.5 % (p=0.73)
Decomposer guild	14.4 %	8.9 %	76.5 % (p=0.77)	14.5 % (p=0.99)

Table 2. Significance of treatment effects on predator guild from pitfall trapping according to Monte-Carlo permutation tests, 1999 permutations.

	Before treatment	Days after treatment		
		1	30	60
Control vs *B. bassiana*	P > 0.16	P > 0.88	P > 0.62	P > 0.34
Control vs lambda-cyhalothrin	P > 0.10	P = 0.02	P = 0.02	P = 0.05
B. bassiana vs lambda-cyhalothrin	P > 0.76	P = 0.02	P = 0.02	P = 0.10

Table 3. Significance of treatment effects on predator guild from soil cores and non-target herbivore guild (post-treatment sampling date) according to Monte-Carlo permutation tests, 1999 permutations.

Predator guild II	P
Control vs *B. bassiana*	0.77
Control vs lambda-cyhalothrin	0.02
B. bassiana vs lambda-cyhalothrin	0.02
Nontarget herbivore guild	
Control vs *B. bassiana*	0.79
Control vs lambda-cyhalothrin	0.27
B. bassiana vs lambda-cyhalothrin	0.80

Figure 1. Persistence of *B. bassiana* spores on foliage (open circles, colony forming units per sq leaf cm^2) and soil (open diamonds, colony forming units per dry soil gram). Daily precipitation is shown (bars, millimeters per day).

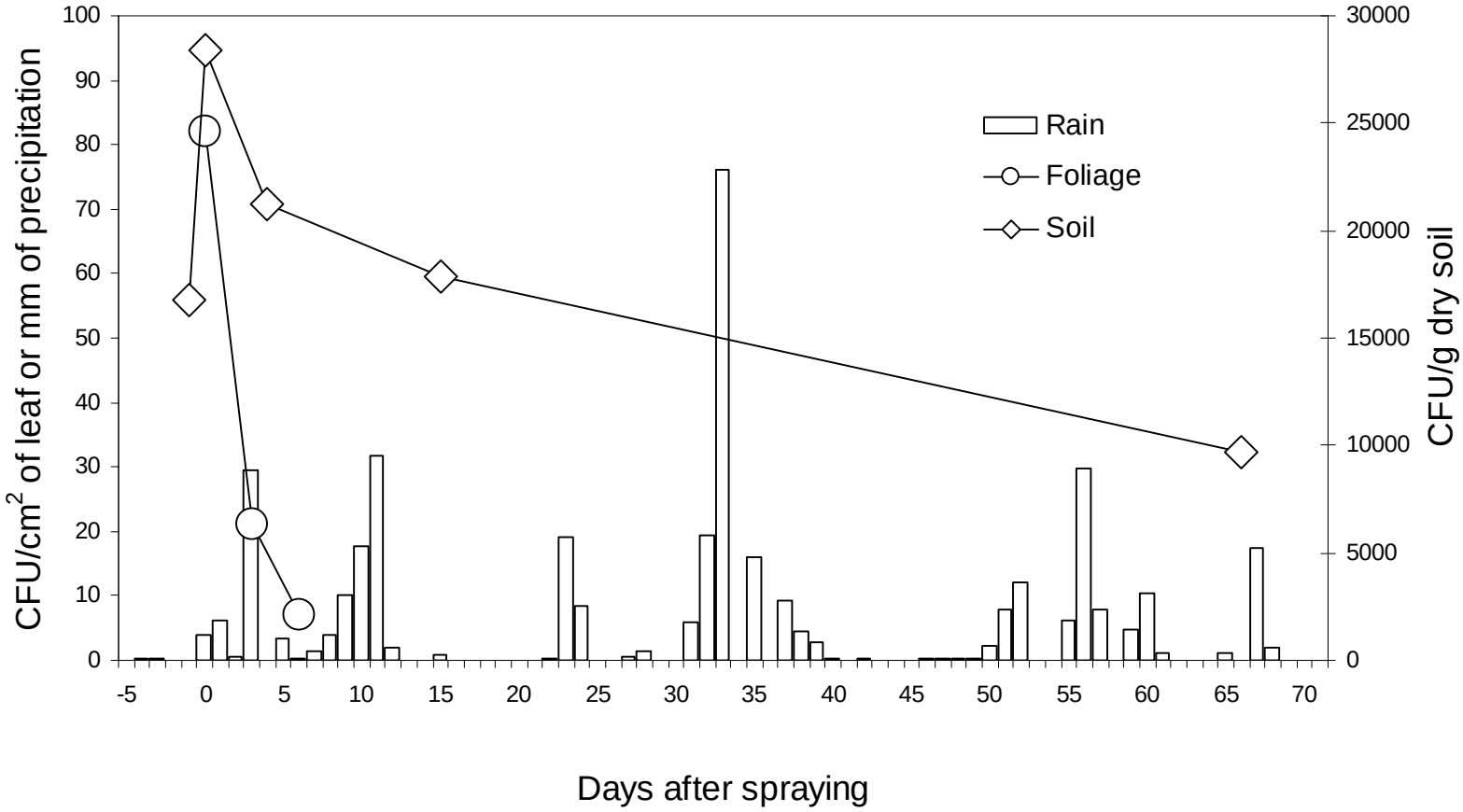


Figure 2. Principal response curve (PRC) for the predator guild from pitfall trapping, indicating the effects of a single spraying of *B. bassiana* (squares) or lambda-cyhalothrin (triangles), compared with the control (circles). Values deviating from the reference value of 0 indicate treatments effects. Weights (right) indicate the affinity of the taxon with the PRC trend.

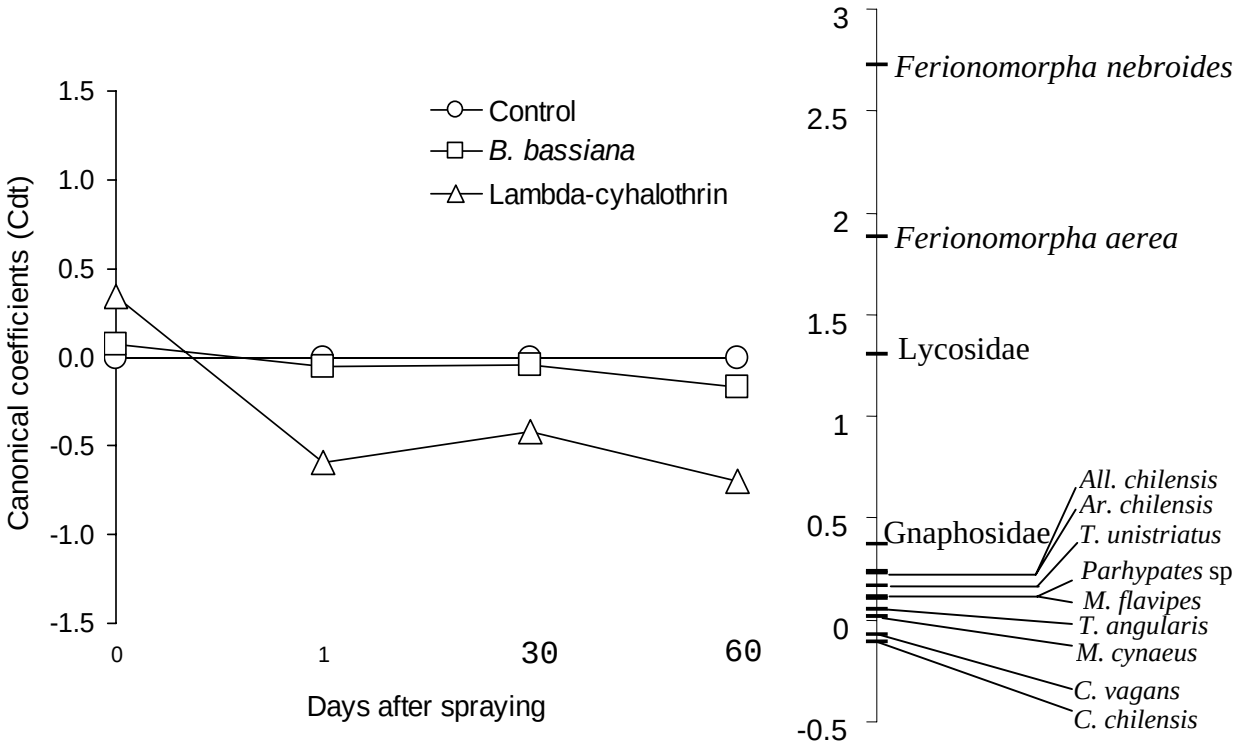


Figure 3. Principal response curve (PRC) for the predator guild from soil cores, indicating the effects of a single spraying of *B. bassiana* (open bars) or lambda-cyhalothrin (grey bars). Weights (right) indicate the affinity of the taxon with the PRC trend.

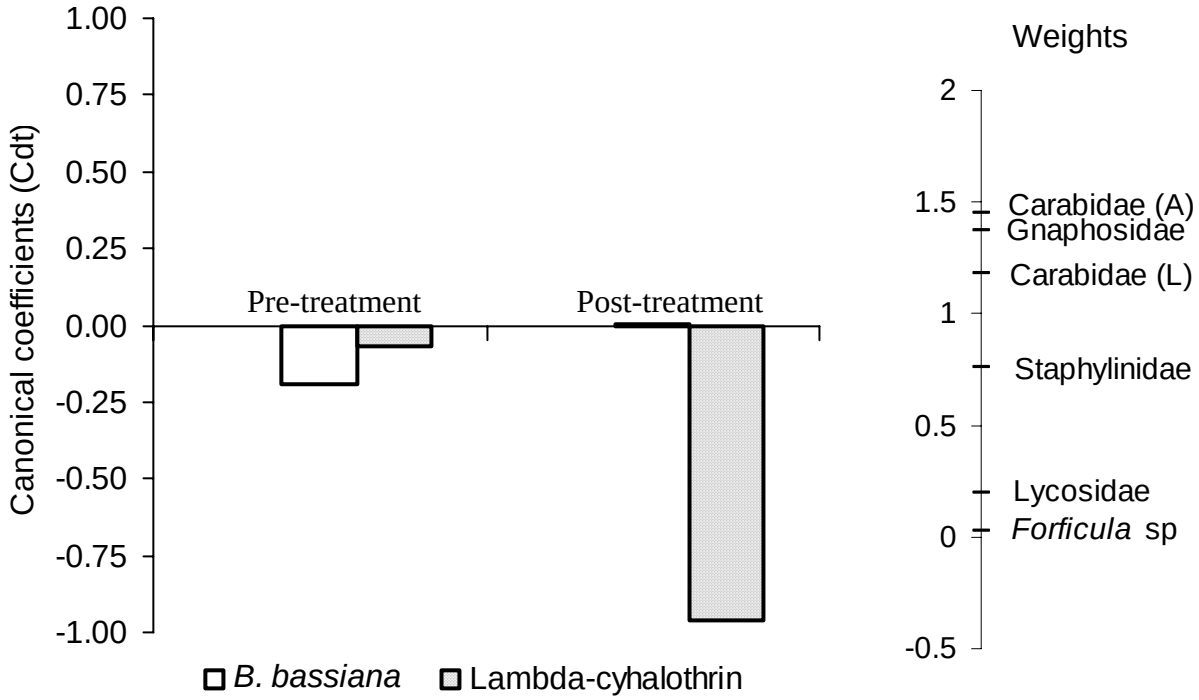


Figure 4. Principal response curve (PRC) for the herbivore guild, indicating the effects of a single spraying of *B. bassiana* (open bars) or lambda-cyhalothrin (grey bars). Weights (right) indicate the affinity of the taxon with the PRC trend.

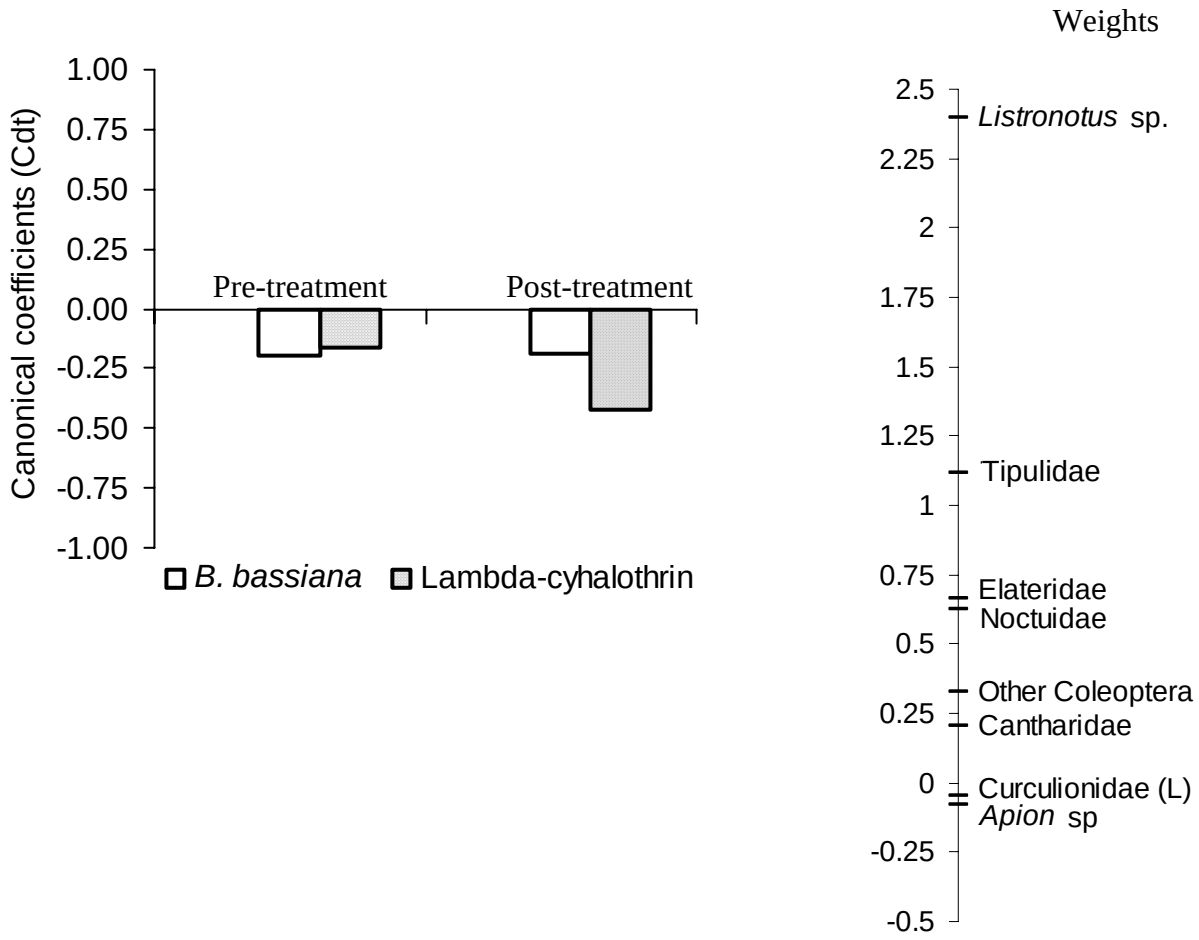
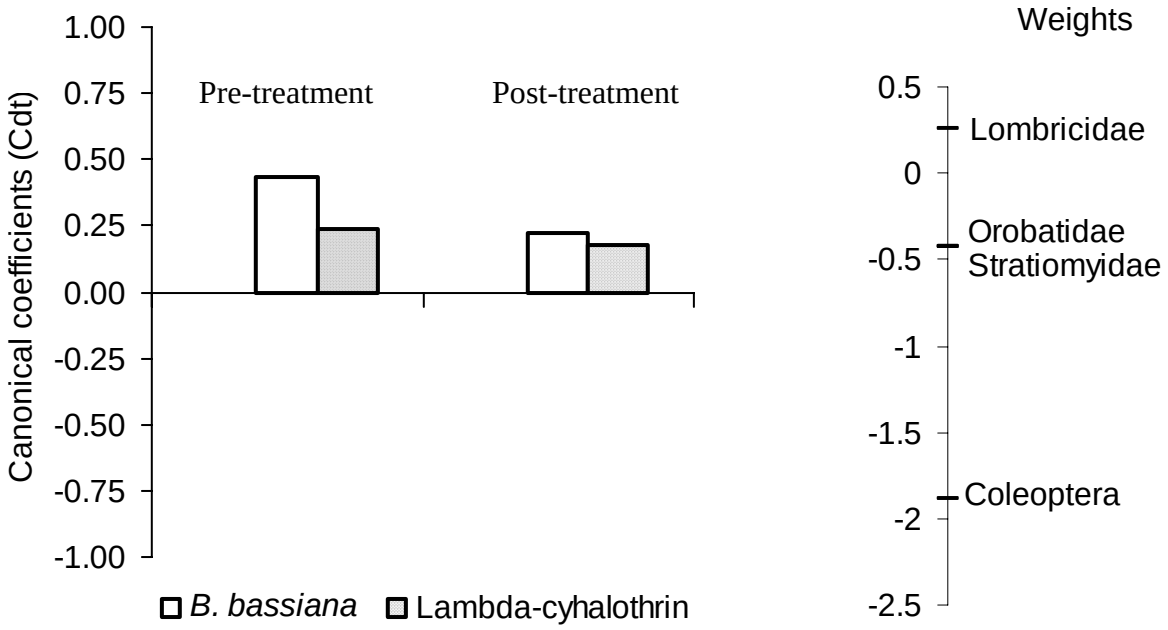


Figure 5. Principal response curve (PRC) for the decomposer guild, indicating the effects of a single spraying of *B. bassiana* (open bars) or lambda-cyhalothrin (grey bars). Weights (right) indicate the affinity of the taxon with the PRC trend.



4.- CAPÍTULO CUARTO: ANÁLISIS DE LOS EFECTOS NO DESEADOS A NIVEL DE COMUNIDAD.

Este artículo puede ser consultado bajo el título:

Devotto L., R. Carrillo, E. Cisternas and M. Gerding, in press. Response of grassland soil arthropod community to biological and conventional control of a native moth: using *Beauveria bassiana* and lambda-cyhalothrin for *Dalaca pallens* (Lepidoptera: Hepialidae) suppression. Accepted in Biocontrol.

RESPONSE OF GRASSLAND SOIL ARTHROPOD COMMUNITY TO BIOLOGICAL AND CONVENTIONAL CONTROL OF A NATIVE MOTH: USING *BEAUVERIA BASSIANA* AND LAMBDA-CYHALOTHRIN FOR *DALACA PALLENS* (LEPIDOPTERA: HEPIALIDAE) SUPPRESSION.

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Abstract.

Conventional and biological control of a native moth, *Dalaca pallens* (Blanchard) (Lepidoptera: Hepialidae), were evaluated in South Chile in relation to changes on community metrics (diversity, species richness, evenness and dominance) of a soil-dwelling invertebrate assemblage. Two experiments were conducted (in winter and spring) to compare non-target effects of *Beauveria bassiana* (Balsamo) Vuillemin and lambda-cyhalothrin insecticide. The invertebrate community was sampled before and after spraying by extracting soil cores. Estimates of diversity (Shannon index), species richness, evenness (Hurlbert's Probability of Interspecific Encounter) and dominance indicated that the invertebrate assemblage was strongly disturbed by lambda-cyhalothrin treatment but not by *B. bassiana* applied in winter, over the sampling period (40 days). Spring results revealed that diversity and evenness at control and at *B. bassiana* plots were similar between them and higher than at lambda-cyhalothrin plots, while there were no differences between sites 30 days after treatment in species richness. Inundative biological control using *B. bassiana* strain QU-B931 was considered to pose lower ecological risk than lambda-cyhalothrin, currently one of the most frequently used insecticides for *D. pallens* control.

Keywords: *Beauveria bassiana*; *Dalaca pallens;* diversity; grassland pests; Hepialidae; inundative biological control; Lepidoptera; rarefaction curves; species richness.

Abbreviations.

PIE: probability of interspecific encounters.

UACH: Universidad Austral de Chile.

A.I.: active ingredient.

µl: microliter.

INIA: Instituto de Investigaciones Agropecuarias, Ministerio de Agricultura, Chile.

Introduction.

Declining diversity has raised concerns for continued provision of ecosystem services (e.g. natural pest control). Intensification of agriculture and the subsequent simplification of agroecosystems has often reduced biodiversity and can result in pest outbreaks (Swift et al., 1996). Conservation of both natural enemies and non-target herbivores is desirable because they play an essential role in whole community dynamics, and within the biocontrol context, they may act as useful predators or as alternative prey when the target species is scarce (Kenmore et al., 1984; Hardin et al., 1995)

Dalaca pallens (Blanchard 1852) (Lepidoptera: Hepialidae) is a native moth from South America (Chile and Argentina) and the most important grassland pest in Southern Chile. Adults emerge in late spring and drop eggs over pastures in flight. The larvae burrow a vertical gallery in the soil and hide in it during daylight. At night, the larvae move to the soil surface consuming leaves and culms. The larval stages develop over 6-8 months in soil, causing extensive loss of forage during this time. About 10% of grassland area (ca. 200,000-300,000 ha) is sprayed against this insect every year, using conventional insecticides such as insect growth regulators and pyrethroids. However, their use is seen as undesirable for biological and economic reasons, therefore alternative strategies are needed to manage this insect and reduce the reliance on chemical insecticides.

Beauveria bassiana Balsamo (Vuillemin) is under study as an efficient, environmentally-friendly and economically competitive biopesticide for *D. pallens* control in Chile. One potentially useful strain has been selected after conducting *in vitro* mortality assays and field studies, which showed that the fungus caused mortality rates similar to the insecticide cyhalothrin-lambda, when sprayed at 10^{12} spores per ha (Cisternas et al., 2003). Farmers have begun to adopt this new technology and the sprayed area increased from 20 ha in 2003 to 300 ha in 2005. However, effects on non-target invertebrates are unknown, as is this treatment's potential conflict with other biological control agents. Several studies have reported side effects of *Beauveria* spp. on non-target arthropods, including carabid larvae (Traugott et al., 2000), braconid and encyrtid wasps (Danfa and van der Valk, 1999) and coccinellid predators (Jayanthi and Padmavathamma, 1996). Preliminary assays on the Chilean species *Allendia chilensis* (Col.: Carabidae) and *Phytoloema herrmanni* (Col.:

Scarabaeidae) have shown that the fungus has the potential to infect and kill non target species, at least in the laboratory (Devotto et al., 2003).

The studies mentioned above were conducted on selected beneficial species, but less work has been done to compare the effects of *D. pallens* control strategies at the community level. This knowledge and adequate assessment of the ecological risks of *B. bassiana* are needed before scaling to extensive use, to prevent unintended adverse effects on the grassland ecosystem functioning, including natural pest control. Regardless of the habitat or ecosystem, arthropods contribute well over half of the metazoan species (Dennis, 2003), playing different roles, including herbivore regulation, nutrient cycling and other ecosystem processes. In relatively simple systems such as grasslands, arthropods even have a higher preponderance.

The aim of this study was to compare the effects of conventional and biological control of *D. pallens* on the grassland arthropod community of South Chile. We designed two experiments to test the hypothesis that *B. bassiana* biopesticide would be less disruptive to the ground-dwelling arthropofauna than the insecticide lambda-cyhalothrin.

Materials And Methods.

Sites and environmental data.

Two trials were conducted: one in winter and one in spring. The winter trial was conducted on a farmer's field near Osorno, Chile (40º35' S, 73°10' W), from July to September 2003. Plant cover was composed of hybrid ryegrass (*Lolium perenne* x *Lolium multiflorum;* 80-90 %) and broad-leaf species, including *Plantago* spp and Cyperaceae (<10%).

The spring trial was conducted at Universidad Austral Experimental Station, near Valdivia, Chile (39°30' S and 73°00' W), from October to Dcember 2003. The plant cover was mostly composed of narrow leaf species (*Lolium* spp. and *Holcus lanatus*; about 80%), white clover (*Trifolium repens*; 10-15 %) and broad-leaf weeds (5-10%). The 4 ha field was bordered by a forest (one side), a road (one side) and two pasture fields (two sides). Temperature and rain data were recorded throughout the duration of the two trials and are shown in Figure 1.

Fungus.

Beauveria bassiana strain QU-B931 was isolated from a field-collected larva of *Dalaca pallens* in 1998, using a semi-selective media described by Alves et al. (1998), based on Chase et al. (1986). Mycelium and spores were cryopreserved at –196 °C and held at the Entomopathogenic Organisms Collection (Instituto de Investigaciones Agropecuarias, Chillán, Chile) and mass-reared in rice bags. Spores were harvested, dehydrated and stored in vacuum. Germination assays were done in both experiments as follow: a sample of the tank mix was taken. In the laboratory, 0.5 ml of the tank sample were spread on 2% agar plates. Plates were held in darkness for 48 h and germination of the spores was recorded at 24 and 48 h counting at least 100 spores in each plate under microscope. Germination percentage was calculated as (number of germinated spores/ total number of spores) x 100. 24 and 48 h counts were averaged as they yielded similar estimates. These analyses gave >80% viability in both cases.

Procedures.

Three sites were established for the winter experiment: control site (0.5 ha); *B. bassiana* site (1 ha) and λ-cyhalothrin (alpha-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate) site (1 ha). Sites were 100-150 apart each other and surrounded by grassland. Treatments were applied on July 24, 2003, a cloudy day, between 09:00 – 13:00 h. Ten grams of conidia were mixed with non ionic detergent (Down, Procter&Gamble, Buenos Aires, Argentina) and suspended in 200 l of water. This suspension was sprayed on the *B. bassiana* site (10^{12} spores per ha), hereafter referred as *B. bassiana* treatment. Lambda-cyhalothrin (ZERO 5 EC, ANASAC, Santiago, Chile) was applied according to the procedure described above (but non ionic detergent) at label rate for *Dalaca* sp. control (7.5 g A.I. per ha).

In the spring experiment, twelve plots (30 x 30 m) were established in the 4 ha field, delimited by wooden sticks. Four plots were assigned to each treatment at random and four plots remained as control. Spores or λ-cyhalothrin were sprayed on October 15, 2003, as described above, under clear skies and no wind, between 18:00 and 20:00 h. The first post-treatment rain fell the following morning. No additional plant protection products were applied. Spray deposition was assessed in the spring experiment: soil and foliage samples were taken and colony forming units (CFUs) per square centimeter of leaf or gram of soil

were estimated using a dilution plating method on agar-oat-dodine selective media (Chase et al., 1986).

Spore persistence in soil. Eight soil cores (9 cm diameter, 10 cm deep) were extracted at random in each plot assigned to *B. bassiana* treatment. They were pooled and put in a plastic bag to get ca. 1-1.5 Kg of soil and cooled (5-10° C) prior to analysis. In the laboratory, the soil was sieved and litter and roots were removed. Fifteen grams of fresh soil were added to a flask with 25 ml of sterilized distilled water and drops of Tween-20 as surfactant. In parallel, three samples of 100 g of soil were dried in a stove to measure the soil water content and express the number of colonies on a dry soil basis. The mix was shaken by hand for 5 min. An aliquot (2.5 ml) was transferred to a second tube and then sterilized water and Tween-20 were added to complete 25 ml. The new tube was treated as above and when all the dilutions were available (10^{-1} to 10^{-3}), we transferred 150 µl of suspension, using a pipette, to each plate with selective media (3 plates per dilution). The plates were cultivated for ten days (no light, 20° C) and colony forming units (CFU) were recorded at the end of this period. A proportion of the colonies was sampled and correct identification was confirmed by microscopic exam. Counts were corrected by water content to express the spore numbers on a dry soil basis. Spore number was estimated five times in the study: before and 1, 5, 15 and 66 days after spraying.

Persistence of spores on leaves. Foliage samples were collected at random in each plot (10-15 points) and pooled. In the laboratory, pieces of leaves were cut with scissors, measured with a rule and added still to complete 32 cm^2 per plot. Leaf pieces were added to a tube with 25 ml of sterilized distilled water and drops of Tween-20. Then, the same procedure of soil samples was adopted. Spore numbers are expressed by fresh leaf area. Only ryegrass leaves were included in this analysis. Sampling was performed at 1, 4 and 7 days after spraying.

The dilution/transference process was repeated 3 times, therefore dilutions from 10^{-1} to 10^{-3} were available, both soil and foliage samples. Only estimation from dilution 10^{-2} were used to draw the soil persistence curve and the foliage persistence curve was drawn from 10^{-1} dilution data (Figure 1).

Data collection.

The abundance and diversity of invertebrates were monitored by extracting soil cores three times in winter (before spraying and 20 and 40 days after spraying) and twice in spring (before spraying and 30 days after spraying): without removing the plant cover, a bucket auger was pressed down to the soil to collect samples from the top 10 cm of soil. Soil cores (9 cm diameter, 10 cm high) were extracted and bagged to prevent desiccation and animal escape. In the winter experiment 120 cores were taken from every site and 30 cores were taken from each plot in the spring experiment.

In the laboratory, 70% of the cores were put on extraction trails and the remaining 30% were put on Berlese-Tullgren funnels (Carrillo et al., 2003), active for 96 h. These proportions were kept constant across sites and dates, thus counts from both extraction methods were pooled. All material from funnels and trails was sieved onto a screen cloth and invertebrates were poured into a Petri dish. Specimens were examined under a stereoscopic microscope, counted and classified to the lowest possible taxonomic level. Identification was performed using keys and illustrations provided by CSIRO (1991) and Artigas (1994) as well as comparing them with those already identified in the Entomology Laboratory Collection, Universidad Austral de Chile (UACH). Arthropods were stored in 70% ethanol and representative specimens were mounted and deposited in the UACH Insect Collection.

Data analysis

Species richness curves, Shannon index, Hurlbert's PIE and dominance were calculated using ECOSIM software (Gotelli y Colwell, 2001; Gotelli and Entsminger, 2004). We set up 1000 iterations for generating 95% confidence intervals (CI) through a Monte Carlo procedure and if calculated CI of two indexes did not overlap, they statistically differed at $\alpha = 0.05$.

The foliage and soil counts of colony forming units (CFU) were submitted to one way ANOVA, with time as factor. Means were compared by Fisher's least significant difference test (LSD, p=0.05). The analyses were performed in S-PLUS 2000 software (MathSoft Inc., Cambridge, USA).

Diversity.

To determine whether treatment affected diversity, we calculated Shannon index as

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i = proportion of individuals represented by each taxon; i = i-th species and S = observed number of species. As Shannon index is sensitive to changes in rare species, we used it in conjunction to other community metrics.

Species richness and rarefaction statistics.

Large differences on abundance were observed between treatments. As treatments with more individuals may have artificially inflated species richness, we employed rarefaction statistics to compare species richness between sites while controlling for abundance differences (Hurlbert 1971). Because of differences at the taxonomic resolution of groups, we will use species richness and taxa richness interchangeably.

Species richness was expressed as the number of expected species (E(S)) within a sub-sample of n specimens. The size of the sub-sample (n) used for comparing treatments was equivalent to the least abundant treatment at every sampling date.

Evenness.

We applied Hurlbert's probability of interspecific encounters (PIE; Hurlbert, 1971) as an evenness measure, which establishes the probability of encounters between two individuals of different species, assuming that every individual in the collection can encounter all the other individuals. This index has a low sensitivity for rare species and gives more importance to evenness of distribution of individuals between species (Barbieri et al., 1999), thus it provides information complementary to the Shannon index. In addition, PIE is unbiased by sample size and number of species in a sample, unlike most other evenness indexes. We used the species-richness module of ECOSIM (1000 iterations) to test differences in arthropod evenness (Gotelli and Colwell, 2001) as

$$PIE = \left[\frac{N}{N+1}\right]\left[1 - \sum_i \left(\frac{N_i}{N}\right)^2\right]$$

N is the total number of individuals and N_i the number of individuals in the i^{th} species. The abundance levels for simulation were fixed according to the treatment with lowest abundance to allow comparison between treatments.

Dominance.

Dominance (fraction of the total catches represented by the most abundant species) was calculated using species diversity module of ECOSIM and 95% confidence intervals were obtained as described above.

Results

Taxonomic and functional identity.

A total of 9555 invertebrates were identified in both experiments. Five taxa accounted for 84% of the total captures: Cantharidae (34%), Acari (30%), Curculionidae (9%), Carabidae (7%) and Araneae (4%). The functional groups represented were herbivores (50%), detritivores (34%), predators (14%) and omnivores (2%). An overview of the community composition is given in Tables 1-5 (anexo 3).

Effects on community metrics.

Diversity.

The diversity changes on winter experiment are shown in Figure 3 (left). Before treatment, diversity at the control site (expressed by Shannon index) was higher than both *B. bassiana* and λ-cyhalothrin sites, while no significant differences were observed between these treated sites (Figure 3, left). Twenty days after a single spraying of *B. bassiana* QU-B931 or λ-cyhalothrin, control and *B. bassiana* sites showed no differences ($\alpha = 0.05$), while the diversity was significantly lower in the λ-cyhalothrin site (Figure 3, left). The same result was observed 40 days after spraying.

The diversity changes in the spring experiment are shown in Figure 4 (top). Samples taken before spraying indicated that diversity in untreated and treated sites was similar. Thirty days after spraying, estimates of Shannon index showed no differences between control and *B. bassiana* sites, while diversity at λ-cyhalothrin site was significantly lower than both control and *B. bassiana* sites.

Species richness.

Rarefaction curves of species richness were calculated for both trials (Figures 5-6). In the winter experiment, species richness estimated at n = 495 did not differ between sites before treatment (Figure 5, A), in spite of marked differences between site abundances (numbers at the *B. bassiana* site were twice-fold those at the control site). In both post-spraying sampling dates, species richness at the control site felt slightly out of the confidence interval calculated around *B. bassiana* rarefaction curve, while λ-cyhalothrin rarefaction curve felt out the lower confidence limit of *B. bassiana* rarefaction curve (Figure 5, B-C). Spring results indicated that the control and λ-cyhalothrin species richness were similar and higher than the *B. bassiana* species richness before treatment, at n = 402 (Figure 6). After treatment, the confidence limits of *B. bassiana* and λ-cyhalothrin overlapped and included the control species richness, therefore the treatments did not alter species richness in this season.

Evenness.

In the winter experiment, evenness before treatment at the control site was higher than evenness at both *B. bassiana* and λ-cyhalothrin sites but there was no significant difference between these latter two sites. Twenty days after a single spraying of *B. bassiana* or λ-cyhalothrin, estimates of Hurlbert's PIE showed a different pattern: control and *B. bassiana* sites showed no differences ($\alpha = 0.05$), but evenness at λ-cyhalothrin site was significantly lower (Figure 3, centre). The same result was obtained when evenness values were calculated from samples taken 40 days after treatment (Figure 3, center).

Pre and post-spraying values for evenness in the spring experiment are shown in Figure 4 (centre). Samples taken before spraying indicated that evenness in untreated and treated sites was similar. Thirty days after spraying, estimates of Hurlbert's PIE showed slight, although statistically significant, differences between control and *B. bassiana* sites and a more marked decreased evenness at λ-cyhalothrin site (Figure 4, centre).

Dominance.

In the winter experiment, dominance was similar between treated sites, but it was lower at the control site (Figure 3, right). Twenty days after spraying, dominance remained high at the λ-cyhalothrin site (where the most dominant species accounted for more than 60% of

catches), while dominant species represented just 45% and 39% of catches at the *B. bassiana* and control sites, respectively (Figure 3, right). At 40 days after treatment, dominance at the control site was slightly higher than dominance at the *B. bassiana* site, but lower than at the λ-cyhalothrin site (Figure 3, right). In the spring experiment (Figure 4), before spraying no species accounted for more than 25% of catches, but post-spray samples revealed that dominance was higher at the insecticide site (50%) than the dominance at the *B. bassiana* (27%) and control sites (33%) (Figure 4, bottom).

Discussion.

Earlier field studies have reported non existent or minimal non-target effects of inundative applications of *B. bassiana* (<10% of abundance reduction) (Brinkman and Fuller, 1999; Ivie et al., 2002; Steenberg et al., 1995; Wang et al., 2001; Wang et al., 2004), in spite of laboratory studies which showed some side effects of *Beauveria* spp. on non-target arthropods (Danfa and van der Valk, 1999; Traugott et al., 2000). The strain *B. bassiana* QU-B931 was evaluated in the laboratory for non target pathogenicity: one non-target white grub (*Phytoloema herrmanni*) and one carabid beetle (*Allendia chilensis*) were tested (Devotto et al., 2003). While no significant differences in mortality were observed between control and treated insects, some specimens were parasitized by the fungus, which raised some concerns about potential adverse effects on non-target species. Although experiments were not designed to test specific hypotheses on single species, data showed that this strain selected against *D. pallens* did not pose a significant risk to the rest of invertebrate community, at least in the short term. Corrected mortality of the target pest was 48% and 68% in the *B. bassiana* winter site, 15 and 30 days after spraying (Cisternas et al., 2003), therefore spores were active and infective despite the low temperatures. In spring, spores were active for at least five days in foliage and seven days in soil (Figure 1), but despite this the pest population did not decrease. We attribute this lack of spring efficacy to the condition of the larvae (full mature larvae are several times heavier than autumn larvae and their feeding activity tends to decrease as they approaches to pupate) rather than to a lack of pathogenicity of the fungus.

Use of λ-cyhalothrin is appropiate against many pest species in major insect orders, both indoor and outdoor environments. This insecticide has a relatively low specifity and several beneficial groups are affected according to laboratory and field studies (Niehoff et al.,

1994; Nowak et al., 2001; Tillman and Mulrooney, 2000; van den Berg et al., 1998), as well as other organisms like fish and non-target herbivores. These studies reported transient decreases in non-target numbers, specifically predators and parasitoids, immediately after λ-cyhalothrin treatment, but numbers recovered within a short period (usually 10-15 days). Our results suggest more long-term effects because the adverse effects of the insecticide were present for at least 40 days after treatment despite of the absence of barriers to movement and the fact that plots were surrounded by untreated grassland providing large sources of migrants. On the other hand, we consider that development stage of the invertebrates could explain, at least partially, these results: in the winter experiment, many individuals were immature and would have lower mobility than adults, therefore the recovery time in this season may be longer than in the spring or summer, seasons in which the most of the mentioned studies were conducted.

The insecticide affected all community metrics in winter, while it affected diversity, evenness and dominance but not species richness in spring. The latter result must be interpreted cautiously, because the period between spraying and sampling in the spring experiment could have been long enough to allow migrants move from surrounded area to plots. Therefore, a negative impact of insecticide in the spring species richness could be masked by movement of these migrants.

In relation to new emerging technologies, for example narrow spectrum insecticides, *B. bassiana* based biopesticides could play an important role because Chilean daily and beef industry can not compete with the rest of South America. Instead, it is focused in high-profit market niches such as organic or naturally raised cattle, where these new pesticides would not be allowed, in spite of they have better properties than the broad-spectrum insecticides currently used. In addition, if an IPM program is implemented for *D. pallens* control, many alternatives will be needed to design an robust and sustainable program.

Linking between biodiversity and ecosystem functioning is accumulating support, but the issue remains unresolved (Ehrlich and Wilson, 1991). This relationship has been hypothesized to be linear (the rivet hypothesis, Ehrlich and Ehrlich, 1981), asymptotic (the redundancy hypothesis; Walker, 1992) or idiosyncratic (Lawton, 1994). Despite of these different theories, it can be argued that some level of biological diversity is necessary to maintain ecological function and resilience (Spratt 1997) but unfortunately, incomplete

knowledge of South Chile grassland functioning precludes us from identifying which fraction of the total diversity is needed to saturate most of the processes present in this agroecosystem. Therefore, until the trophic relationships between the different species could be revealed, we advocate for a conservative approach to diversity loss.

Our studies were not designed to test hypotheses about specific functional groups or species, thus we can not identify the mechanism(s) that explain(s) the observed decreased diversity. In spite of this, we raise concerns about continued use of these kind of insecticides because several cases of natural regulation disruption have been recorded in intensified agricultural systems much before explicit mechanisms that explain natural herbivore regulation had been revealed (Hardin et al., 1995). In fact, there are several well documented cases of release of herbivore insects from control after removing their natural enemies (Hills and Taylor, 1951; Kenmore et al., 1984) or non-target herbivores acting like alternative prey when the pest is scarce and contributing to support early season build-up of generalist natural enemies (Settle et al., 1996). In an oversimplified system like pastures, any additional biodiversity loss could exacerbate this process, reduce the system stability and increase the reliance on external inputs, especially insecticides, to maintain its productivity.

It remains a challenge to identify the several roles that the different species play in the pasture. However, as it was mentioned above, negative effects on ecosystem processes with declining biodiversity could be serious much before knowledge about explicit mechanisms could be gathered.

Growers must be aware of how their pest practices may influence ecological processes before to make management decisions. Inundative biological control of *Dalaca pallens* using *Beauveria bassiana* spores did not affect grassland diversity and its components (species richness and evenness), at least in the short term. Lambda-cyhalothrin, one of the most common insecticides used for *D. pallens* control, affected all the community metrics (diversity, richness, evenness and dominance), with unknown consequences for the grassland agroecosystem. It was clear that the community-level impact of this broad-spectrum insecticide was far greater that any effect of *Beauveria bassiana* on arthropod community.

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Figure 1. Temperature and rain data for winter and spring sites (2003).

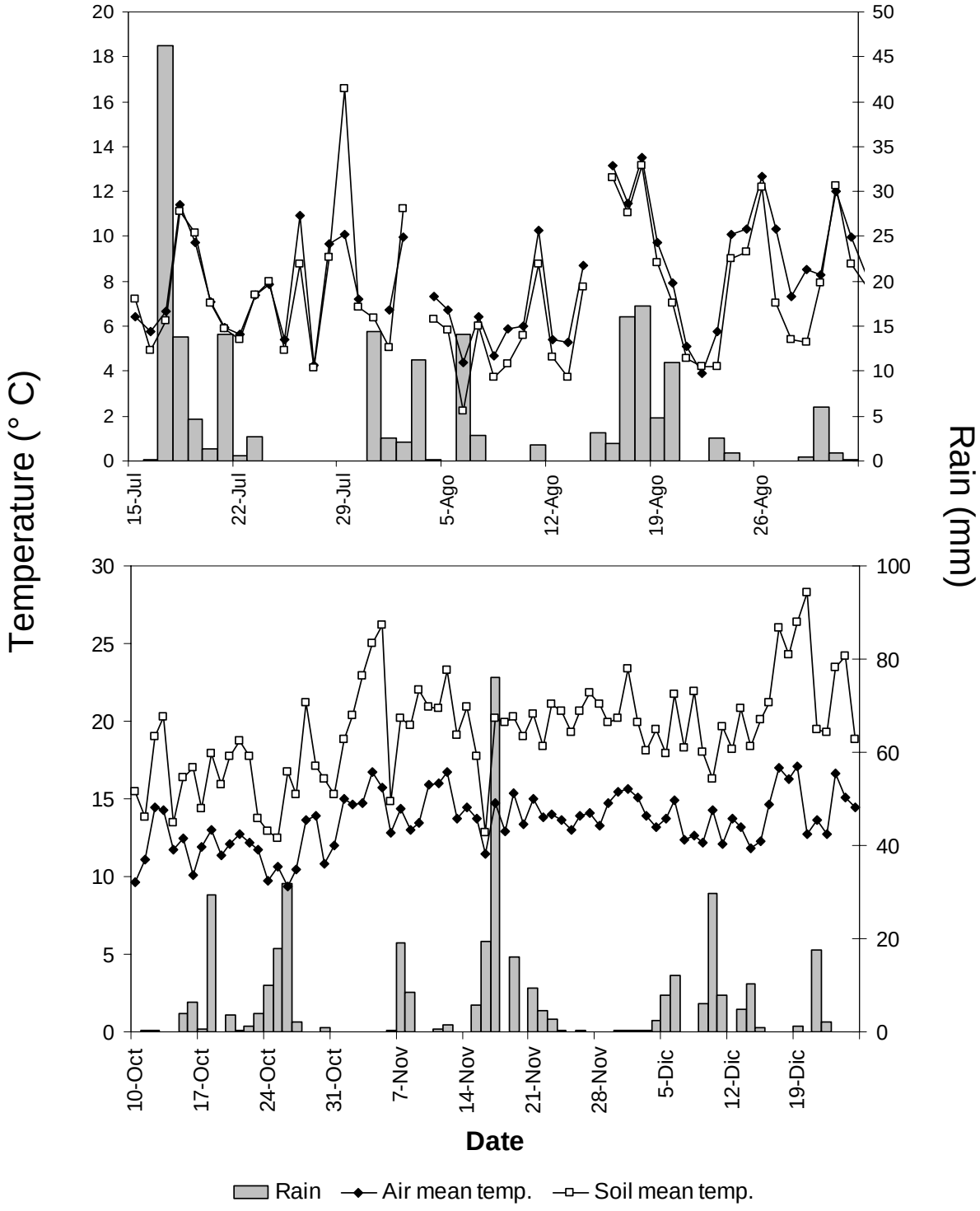


Figure 2. Estimated numbers *Beauveria bassiana* spores in soil and pasture foliage. On each curve, means followed by different letters differ according to Fisher's least significant difference test ($p<0.05$).

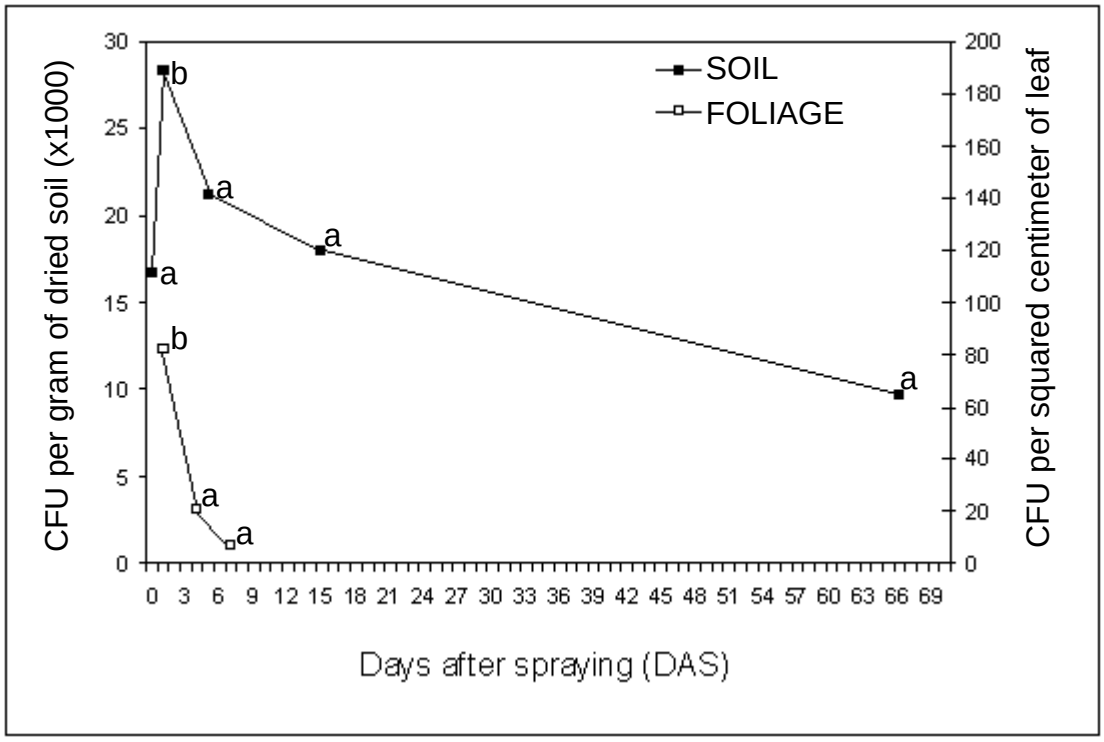


Figure 3. Effects of *Beauveria bassiana* and lambda-cyhalothrin insecticides on diversity (Shannon index), evenness (Hurlbert's PIE) and dominance (proportion of the most common species) before (A), 20 (B) and 40 (C) days after spraying (winter trial). Error bars indicate 95% confidence limits over 1000 iterations (Gotelli and Colwell, 2001).

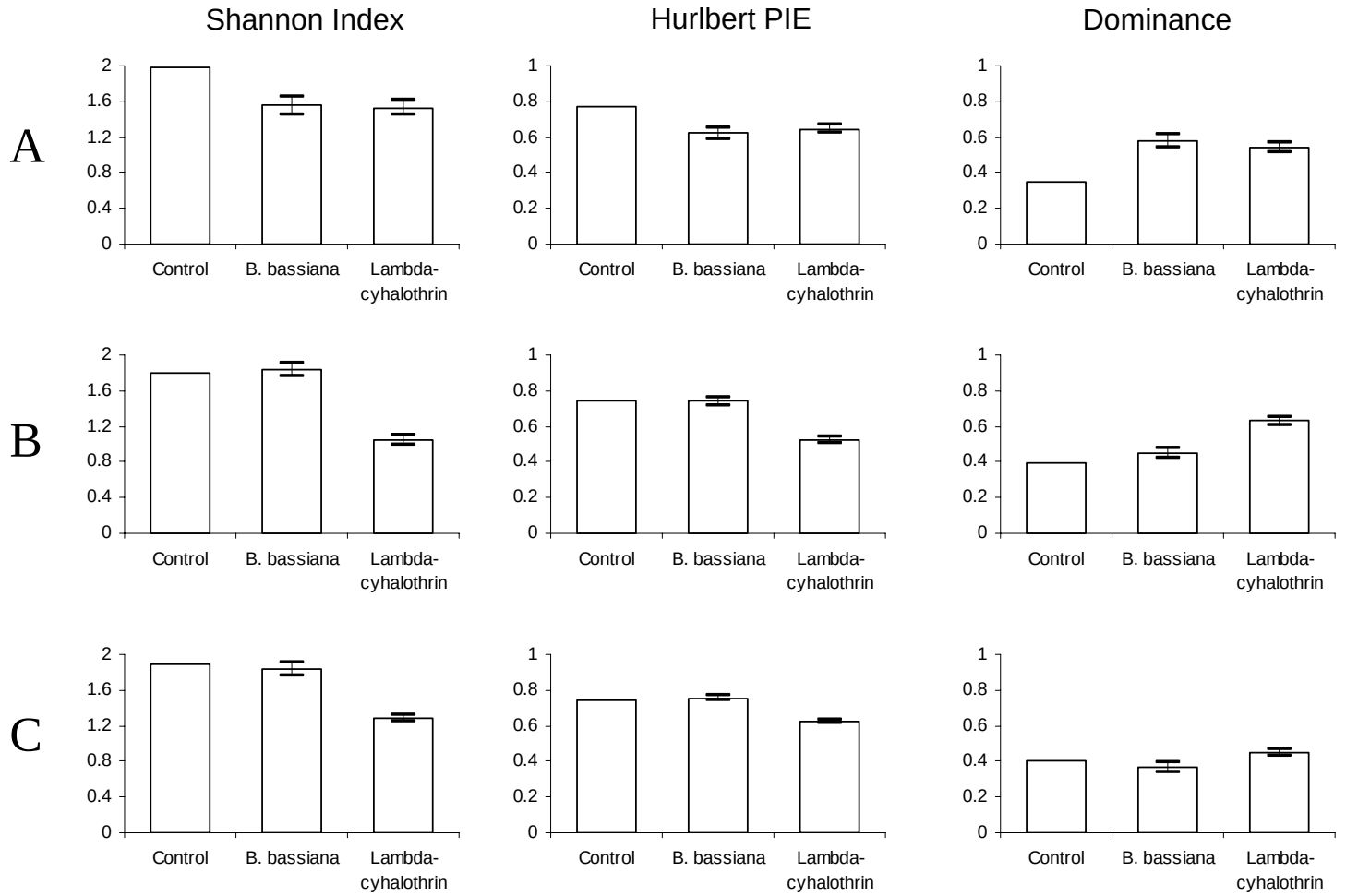


Figure 4. Effects of *B. bassiana* and lambda-cyhalothrin insecticides on diversity, evenness and dominance (spring trial): Shannon index, Hurlbert's PIE and proportion of the most common species before (left) and 30 days after spraying (right). Brackets indicate 95% confidence limits over 1000 iterations (Gotelli and Colwell, 2001).

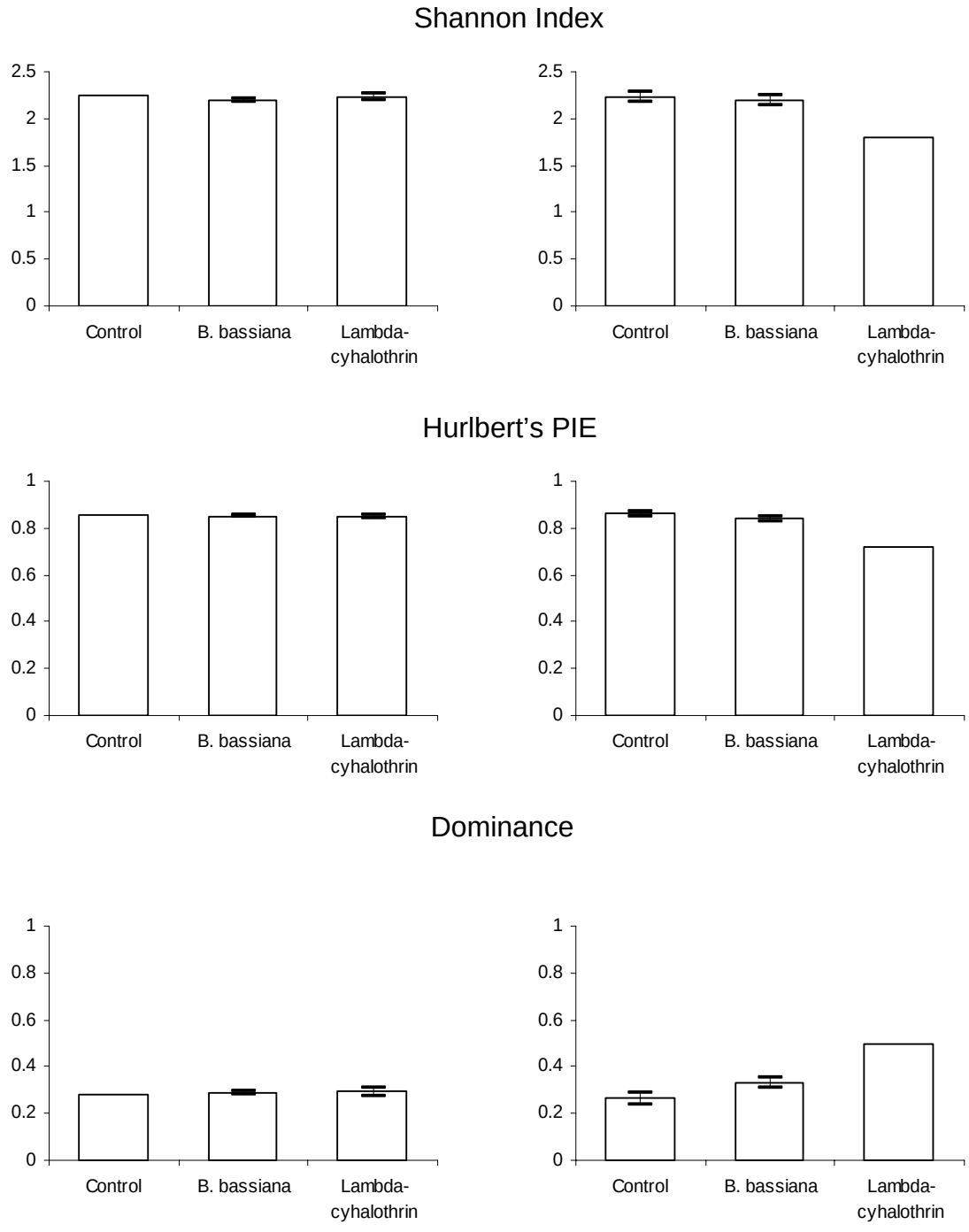
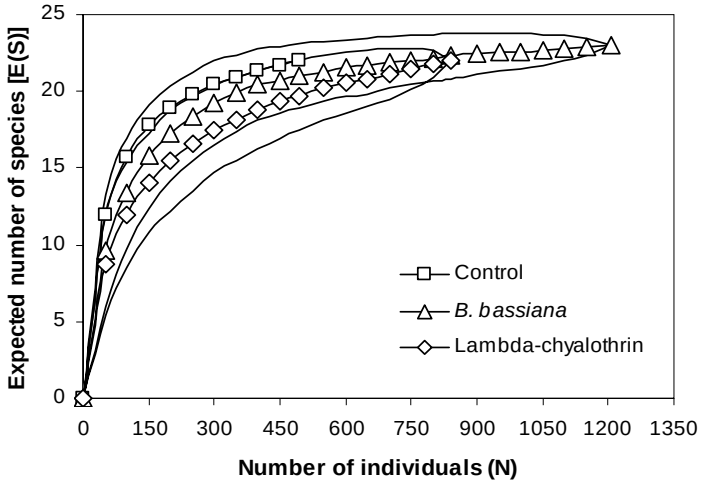
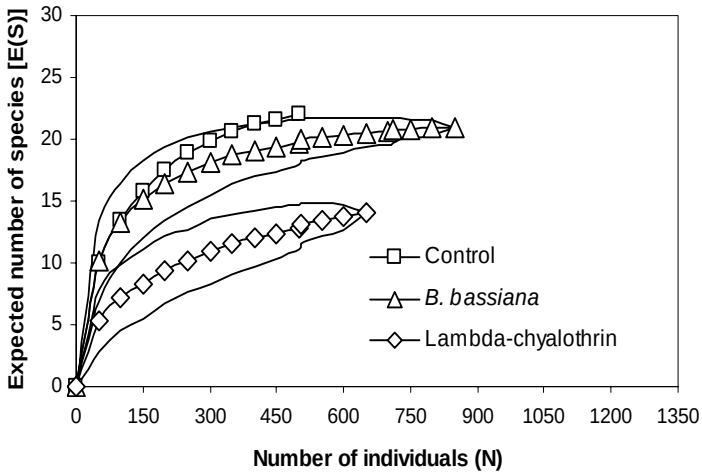


Figure 5. Rarefaction curves for the species richness of invertebrates of a grassland soil assemblage at three dates (winter experiment): A = pre-treatment; B = 20 days after treatment and C = 40 days after treatment. Continuous lines above and below *Beauveria bassiana* and lambda-cyhalothrin curves are 95% confidence limits calculated over 1000 iterations (Gotelli and Colwell, 2001).





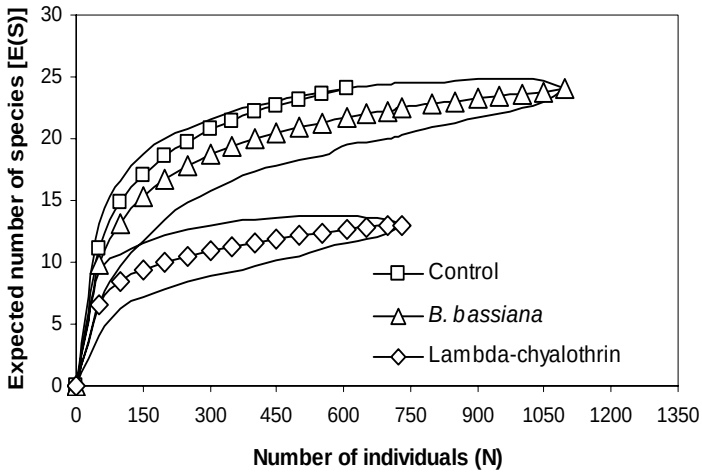
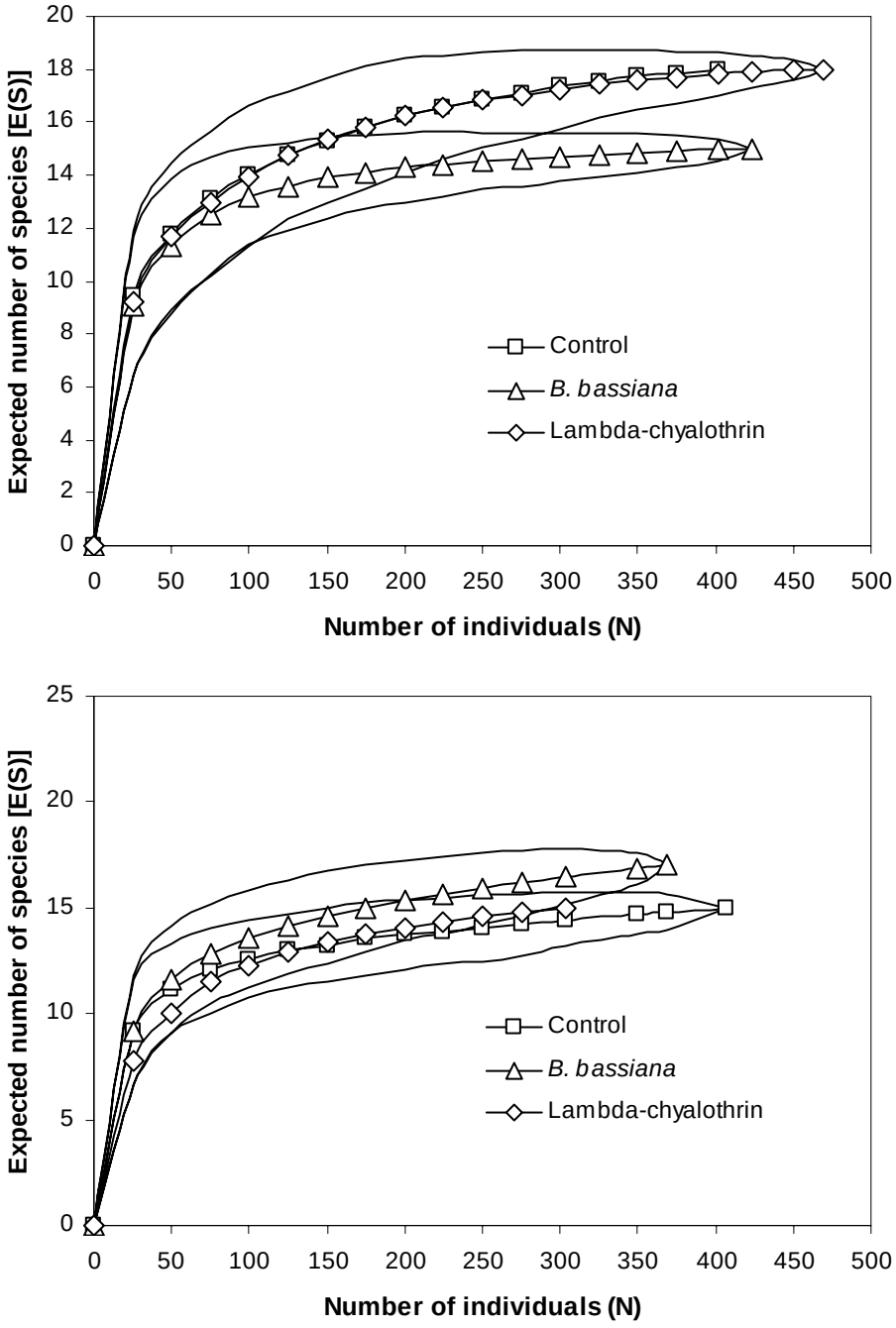


Figure 6. Rarefaction curves for the species richness of invertebrates before (above) and after (below) treatments (spring experiment). Continuous lines above and below curves are 95% the confidence limits calculated over 1000 iterations (Gotelli and Colwell, 2001).



DISCUSIÓN.

Diversidad genética de *B. bassiana* en Chile.

El análisis basado en la región intergénica nuclear conocida como B loc reveló que *B. bassiana* está representada en Chile por un alto número de grupos genéticos, puesto que 20 haplotipos fueron encontrados sobre una muestra de casi cien aislamientos, muchos de ellos separados por miles de kilómetros. A juicio del autor, tres son los hechos más relevantes después de realizar este análisis: la ausencia de una clara estructura genética de acuerdo al origen geográfico de los aislamientos; una mayor diversidad genética en la zona central del país; y una aparente separación de los distintos tipos genéticos en dos grandes grupos.

Respecto del primer punto, el 90% de la variación genética está presente al interior de las poblaciones y sólo el 10% de la variación se produjo entre las poblaciones previamente definidas en este estudio. Si bien estas poblaciones fueron definidas en base a criterios geográficos y climáticos, esta definición es necesariamente arbitraria y depende del juicio del investigador. Existe la posibilidad que existan otros factores, distintos a los usados para definir las poblaciones, que puedan influir en la estructuración genética de este hongo. Mientras algunos haplotipos estaban restringidos a ciertas poblaciones (dos haplotipos son exclusivos de Isla de Pascua, mientras otro fue encontrado sólo en la zona central de Chile), el haplotipo más numeroso a su vez estaba ampliamente distribuido desde el extremo norte hasta el extremo sur, incluyendo Isla de Pascua. La presencia de este haplotipo en lugares tan disímiles plantea varias posibilidades: la capacidad de dispersión de este hongo podría estar sub-valorada; esta especie podría tener una alta plasticidad fenotípica o este haplotipo podría ser un estado ancestral en Chile. Esta última posibilidad no es apoyada por los datos, ya que este haplotipo no ocupa una posición central (ver figura 3, capítulo I) ni los demás haplotipos derivan de él, como podría esperarse si este haplotipo fuera ancestral.

Los extremos del país (Tarapacá, Aysén y Magallanes) presentaron una diversidad genética mucho menor en comparación a la zona central del país. La zona de mayor diversidad

genética coincide con la zona de mayor actividad agrícola, mientras que las zonas con menos diversidad coinciden con áreas con agricultura de subsistencia, de reciente introducción o simplemente con áreas poco intervenidas por el hombre. Basado en lo anterior, la intervención humana podría jugar o haber jugado un papel importante en la dispersión de este hongo.

Algunos autores (Rehner y Buckley, 2003) han sugerido la presencia de especies crípticas en *B. bassiana*. Aunque las regiones secuenciadas son diferentes, la presencia de estos dos grandes grupos apoyaría lo planteado por estos autores, más aún cuando una pequeña submuestra (11 aislamientos) del conjunto estudiado en esta tesis fue estudiado en base a otra región génica (factor de elongación 1 alpha, EF-1α). Al insertar estas 11 secuencias chilenas en el árbol creado por estos autores, parte de ellas quedaron incluidas en el clado conocido como "*B. bassiana*", mientras que la otra parte quedó incluida en el clado llamado preliminarmente "pseudobassiana", el cual es parafilético respecto al primero (Devotto y Rehner, datos no publicados). Hasta el momento, la descripción de nuevas especies basadas sólo en caracteres moleculares no es aceptada por toda la comunidad taxonómica, pero esta situación podría cambiar en el futuro. En el presente, esta información podría servir para revisitar la morfología, fisiología y/o ecología de estos dos grupos aparentemente distintos, para eventualmente encontrar algún carácter que no haya sido advertido hasta ahora y que podría reafirmar la presencia de más de una especie en lo que actualmente se conoce como *B. bassiana*.

Efectos en la artropafauna.

En términos generales, la artropofauna presente respondió diferencialmente al tipo de control utilizado, en ambos sitios de estudio. Estas respuestas fueron detectables en los tres niveles de análisis (taxa, gremio, comunidad) y tendieron a manifestarse por un período mayor en comparación a trabajos similares.

Las técnicas de muestreo utilizadas durante el período abarcado por los experimentos revelaron una comunidad de artrópodos compuesta por casi una treintena de taxa. Estos taxa, de acuerdo a antecedentes de literatura y a su morfología, cumplirían roles muy

diversos dentro del agroecosistema que representan este tipo de praderas, incluyendo taxa considerados depredadores, herbívoros, fungívoros, descomponedores, entre otros. Las tres técnicas de muestreo utilizadas (examen de cuadrantes de suelo, extracción de cilindros de suelo y trampas de caída) tienen claras tendencias a capturar ciertos tipos de artrópodos y por lo tanto estos sesgos deben ser tenidos en cuenta al momento de aceptar o rechazar las conclusiones propuestas. En consecuencia, todas las afirmaciones que se encuentran más adelante fueron realizadas asumiendo que el lector está conciente de lo anterior y que las colocará en una adecuada perspectiva.

Respuesta de los taxa en forma individual.

En el experimento de primavera, un total de once especies de carábidos y dos familias de arañas fueron detectadas durante el período de estudio (60 días). De estos trece taxa, siete estuvieron suficientemente representados para ser analizados estadísticamente en forma individual y de esta forma evaluar el impacto de los dos biocidas utilizados.

Las especies de carábidos más numerosas fueron especies de mediano tamaño (6.5- 14.7 cm), las que predominaron numéricamente frente a carábidos de tamaño menor y mayor al rango nombrado anteriormente. La mayoría de las especies poco representadas en las capturas tendrían máximos de actividad anteriores o posteriores al período de muestreo, de acuerdo al estudio realizado por Zelada (1998) en la zona. En esta categoría estarían especies como *Ceroglossus chilensis, Metius flavipes* y *Pelmatellus* sp. Sin embargo, se debe considerar que tanto este estudio como el de Zelada (1998) son trabajos realizados durante un año y por ende no debe descartarse que alguna de estas especies presente una gran variabilidad de actividad/población entre años, aspecto que podría ser importante al momento de determinar en qué momento de su ciclo estas especies podrían ser más afectadas por el control de cuncunilla negra.

Por otro lado, el uso de trampas de caída secas en lugar de trampas con líquidos preservantes también pudo influir en la composición de las capturas, ya que al permanecer vivos dentro de la trampa, los individuos de mayor tamaño pueden haber depredado a los de menor tamaño y de esta forma explicar la baja presencia de especies tales como

Crosonychus viridis, *Trechisibus angularis* o *Parhypates* sp., especies que de acuerdo a muestreos anteriores están activos entre septiembre y diciembre.

Otro efecto de las trampas secas tiene relación con la eficiencia de captura. En el experimento de primavera, se capturó 1608 individuos, lo que se traduce en un promedio de captura diario por trampa de 0.7 individuos, lo que podría considerarse bajo. Si bien es cierto que tener altas capturas es deseable, en especial desde el punto de vista estadístico, una hipotética baja eficiencia de captura está lejos de afectar la validez de las conclusiones, si no todo lo contrario. Las diferencias entre los tratamientos fueron detectables a pesar de estas hipotéticas bajas capturas y en caso de haber usado técnicas más eficientes, las diferencias deberían mantenerse o acentuarse, pero no disminuir. Una razón adicional para elegir este tipo de trampas es la posibilidad de comparar los resultados con estudios anteriores realizados en la zona o en las cercanías, todos los cuales han usado trampas secas (Zelada, 1998, Morales, 2000).

El período de muestreo se eligió considerando que el insecticida lambda-cyhalotrina tiene efectos que perduran por 2-3 semanas y que la acción de los hongos entomopatógenos en general, incluyendo *B. bassiana*, se completa en un máximo de 45-60 días, siendo usual que el proceso de patogénesis tarde menos que lo indicado. Prácticamente en todos los taxa incluidos en el análisis se observó una tendencia de capturas creciente, lo que refleja la mayor actividad de estas especies a medida que se incrementa la temperatura. En cinco de los siete taxa sometidos a ANDEVA, las capturas un día antes y un día después de la aplicación de los tratamientos fueron muy bajas, lo que impide afirmar si en estos taxa existió un efecto “instantáneo” de los biocidas en la actividad de esos depredadores, en especial si se considera que en cuatro de ellos se detectó efectos negativos en fechas posteriores. En los restantes dos taxa, *F. nebroides* redujo su actividad inmediatamente después del tratamiento, mientras que Lycosidae presentó una tendencia a disminuir que fue marginalmente significativa en esa fecha de muestreo.

Cuatro de los siete taxa sometidos a ANDEVA fueron afectados, al menos en una fecha de muestreo, por la aplicación del insecticida lambda-cyhalotrina. Las reducciones producidas

por este insecticida tendieron a manifestarse a los 30 y 60 días (dos ocasiones en cada fecha) en lugar de 1 día después de la aplicación (una ocasión). Considerando el mecanismo de acción de este insecticida, cabría esperar que las hipotéticas reducciones de actividad se hubiesen manifestado desde la primera fecha de muestreo, pero las bajas capturas en esa fecha habrían impedido detectar eventuales efectos de los tratamientos.

Desde el punto de vista de la magnitud y persistencia de los efectos negativos, el carábido *F. nebroides* fue la especie más afectada por la aplicación de lambda-cyhalotrina. Esta especie redujo su actividad a lo largo de todo el período de estudio, aunque la reducción en la fecha intermedia de muestreo (30 días) no fue significativa al 5%, mientras que la reducción fue mayor al inicio del muestreo (casi 90% de menor actividad) que al final (62% de reducción). Otra especie de carábido (*F. aerea*) vio reducida severamente su actividad a los 60 días después de aplicar lambda-cyhalotrina, puesto que la actividad de esta especie en las parcela testigo era 3.4 veces mayor que en las parcelas con lambda-cyhalotrina.

Las familias de arañas Lycosidae y Gnaphosidae también fueron afectadas por el insecticida, pero el patrón de respuesta de los arácnidos fue diferente al patrón de respuesta de los carábidos. Ambas arañas mostraron una menor actividad 30 días después de la aplicación, pero en la siguiente fecha de muestreo (60 días) ambas familias tuvieron niveles de actividad semejantes al control. Por lo tanto, las arañas fueron capaces de recuperarse dentro del período de estudio, mientras los carábidos no tuvieron esta capacidad. Esta diferencia podría relacionarse con la habilidad de ambos grupos para diseminarse o con la época reproductiva.

En general, los carábidos son reconocidos como buenos caminadores. El tamaño de las parcelas, la presencia de abundantes fuentes de inmigrantes alrededor del sitio de estudio y la ausencia de barreras evidentes son factores que no deberían haber impedido el movimiento de los carábidos. Sin embargo, esta esperada re-distribución de carábidos no se produjo por razones que no están claras.

Existen pocos antecedentes sólidos para anticipar las posibles consecuencias de la reducción de estos taxa en el control de *D. pallens*. Aunque los carábidos son reconocidos

depredadores de lepidópteros, en la época en la que estas especies tienen su máxima actividad, las larvas de *D. pallens* prácticamente han completado su desarrollo y son varias veces más grandes que los carábidos más numerosos. Si a lo anterior se agrega la agresividad y fortaleza de las larvas de *D. pallens*, parece poco probable que las especies de carábidos más abundantes en esa época del año sean depredadores relevantes de *D. pallens*. Carábidos de mayor tamaño, tales como *Calosoma vagans* o *Ceroglossus chilensis*, comenzaron a ser capturados sólo hacia el final del estudio y es difícil establecer si alguno depreda *D. pallens*, en especial cuando algunas de estas especies tienen una digestión pre-oral. Sin embargo, debe considerarse que el género *Calosoma* se conoce comúnmente como "caza-lepidópteros" por su tendencia a depredar larvas de este grupo (French et al., 2004; Toft y Bilde, 2002) y por ende no debe descartarse que *C. vagans* sea un depredador de *D. pallens*.

En apoyo a lo anterior, cabe mencionar que en varias ocasiones larvas de *D. pallens* cayeron en las trampas. Estas larvas fueron atacadas y consumidas por los carábidos, quienes aparentemente compensaron la diferencia de tamaño con su mayor número (observación personal). A pesar de ser una situación artificial y tal vez de difícil ocurrencia en el campo, este antecedente merece ser tenido en cuenta y enfatizaría la importancia de mantener altas poblaciones de estos depredadores.

Hasta el momento, la discusión se ha centrado en la depredación de las larvas maduras, pero los huevos y las larvas neonatas de *D. pallens* son, desde el punto de vista de los depredadores, recursos abundantes en la pradera durante algunos meses del año (desde noviembre hasta marzo). Cada hembra adulta de *D. pallens* es capaz de producir hasta 2000 huevos que son "bombardeados" en la pradera en forma casi aleatoria. Estos huevos y las larvas que de ellos nacen podrían ser depredados por alguno(s) de los taxa estudiado(s) y este aspecto, a juicio del autor, merecería ser investigado y pone una nota de precaución ante la pérdida o reducción de estos depredadores, al menos hasta que se aclaren las relaciones tróficas presentes en la pradera. Estudios en curso (Carrillo, comunicación personal) han demostrado, en primer lugar, que los huevos de *D. pallens* son abundantes en

las praderas (20.000 huevos por m^2) y que, en laboratorio, varias especies de carábidos de tamaño intermedio muestran un alto consumo de huevos de *D. pallens*.

Se ha reportado que las arañas pueden influir en la dinámica de especies a pesar que éstas no sean efectivamente atacadas por aquellas, ya que la presencia de las arañas produciría cambios en el comportamiento de esos herbívoros, los que evitarían a las arañas temporal o espacialmente, a pesar de no ser presas de ellas (Greenstone, 1999). Este hecho realza la importancia de este tipo de depredadores y pone de manifiesto que una eventual reducción de las arañas en las praderas podría beneficiar a insectos herbívoros que actualmente no superan sus umbrales de daño económico.

Los efectos negativos del insecticida lambda-cyhalotrina fueron evidentes a pesar que las parcelas usadas en este experimento no fueron de gran tamaño y que aparentemente no había barreras para el movimiento de los depredadores de superficie. Jepson y Thacker (1990) demostraron una correlación positiva entre el tamaño de las parcelas y la duración del efecto. Por lo tanto, la aplicación de este insecticida en grandes superficies homogéneas, tal como sucede en el sur de nuestro país, podría producir efectos negativos de mayor duración a los detectados en este experimento, por la razón señalada anteriormente. Estos efectos negativos podrían acentuarse si se considera que los carábidos afectados se reproducen en primavera y la reducción de hembras fértiles y/o la reducción de larvas neonatas podría extender el efecto más allá del período de estudio, ya que las especies que sean presa de las larvas durante el verano enfrentarían una menor presión por parte de sus depredadores.

Aunque a menudo un depredador puede ejercer una fuerte interacción negativa sobre una o más de sus especies presa, se está acumulando evidencia de que un gremio en conjunto puede ser más efectivo que sus miembros en forma individual (Koss et al., 2005). Esto ha sido sugerido tanto para carábidos como para arañas y es el fundamento para realizar un análisis a ese nivel (Greenstone, 1999; Sunderland, 1999; Symondson et al., 2002).

Respuesta a nivel de grupos funcionales.

Las curvas de respuesta principal (PRC) construidas para cada uno de los grupos definidos mostraron que los depredadores, independientemente del método de muestreo utilizado, fueron afectados por la aplicación de lambda-cyhalotrina, pero no por la aplicación de esporas del aislamiento QU-B931. Ninguno de los dos biocidas afectó ni a los herbívoros no plaga ni al grupo de descomponedores.

Los resultados de los análisis realizados en forma individual y los resultados de esta nueva metodología coinciden ampliamente, ya que los taxa más afectados según los ANDEVAs tiene a su vez los coeficientes canónicos más altos. Por una parte, este hecho reafirma la consistencia de los resultados obtenidos y por otra, otorga validez a esta técnica de análisis multivariado, cuya aplicación en sistemas terrestres se ha extendido sólo en años recientes. En mi conocimiento, este estudio debe estar entre los primeros estudios que utilizan PRCs para describir el efecto del control de plagas en comunidades de artrópodos epígeos, con claras ventajas frente al análisis univariado tradicional. Estas ventajas se relacionan principalmente con el ahorro de tiempo y de cálculos necesarios para presentar este tipo de resultados en una única figura que sea de fácil comprensión para el lector. Otra ventaja de mayor relevancia es el hecho que las comparaciones entre tratamientos se realizan usando métodos no paramétricos (permutaciones de tipo Monte Carlo), las que no necesitan que los datos se ajusten a alguna de las distribuciones conocidas y no se ven perjudicadas por la gran cantidad de ceros presentes en este tipo de muestreos. Por el contrario, si los datos no son normales y la variabilidad de los datos es alta (incluyendo la presencia de ceros), la aplicación correcta del análisis de varianza tradicional se ve limitada severamente (van den Brink y ter Braak, 1998).

Las trampas de caída y la extracción de cilindros de suelo coincidieron mayormente en las especies de depredadores presentes en la pradera, con excepción de la familia Staphylinidae. Sin embargo, el tiempo y el trabajo que demanda extraer y procesar los cilindros de suelo es mucho mayor que las trampas de caída, con la desventaja adicional que el número de individuos capturados en los cilindros de suelo es mucho menor que los

individuos capturados por las trampas de caída. Por lo tanto, desde el punto de vista de la eficiencia, es más recomendable el uso de trampas de caída para evaluar las poblaciones de depredadores epígeos adultos.

Sin embargo, la situación es distinta cuando se considera otros grupos distintos a los depredadores de superficie, incluyendo a los estados inmaduros de éstos. Los cilindros de suelo fueron particularmente eficientes y efectivos para taxa tales como larvas de Cantharidae, Oribatida, larvas de Carabidae y adultos de Curculionidae. En resumen, el uso combinado de ambas técnicas permitió una mejor evaluación de la artropofauna presente y de su respuesta frente al uso de biocidas.

La aplicación repetida de insecticidas puede cambiar la composición de los ensambles de carábidos en el largo plazo, favoreciendo a unas pocas especies capaces de resistir estas perturbaciones. Las consecuencias de la pérdida de depredadores tales como los carábidos y las arañas licósidas y gnafósidas pueden afectar el correcto funcionamiento del control natural de herbívoros en la pradera, además de reducir la biodiversidad de la familia en nuestro país, ya que la fauna de carábidos chilenos es altamente endémica (55%, de acuerdo a Roig-Juñent y Domínguez, 2001).

La presencia abundante de depredadores generalistas, tales como Carabidae, Lycosidae y Gnaphosidae, podría contribuir a establecer sistemas más balanceados y menos susceptibles a la irrupción de plagas (Mathews et al., 2004). La conservación de gremios completos de depredadores generalistas por sobre alguno de sus componentes, ha sido propuesta como una forma de controlar complejos de plagas diversos y que poseen generaciones en distintas épocas (Brown y Adler, 1989). En relación a una posible reducción de la población de arañas, cabe mencionar que aunque este grupo carece de algunas características altamente deseables en un controlador biológico (respuesta denso-dependiente frente al incremento de sus presas, por ejemplo), ellas se ajustan bien a un modelo de equilibrio (Riechert, 1999) que sí es factible en sistemas relativamente estables tales como las praderas permanentes del sur de Chile.

La conservación de los herbívoros no plaga y de los invertebrados descomponedores puede jugar un rol positivo en el control natural de plagas, ya que en otros sistemas agrícolas intensivos se ha demostrado que estas especies sirven como presas alternativas cuando la especie plaga primaria está ausente y por lo tanto contribuyen a mantener las poblaciones de depredadores (Hardin et al 1995).

Como gremio completo, el grupo de descomponedores no mostró un aumento estadísticamente significativo. No obstante lo anterior, los ácaros Oribatida tuvieron un alto coeficiente canónico, es decir, mostraron una tendencia a aumentar después de la aplicación de los tratamientos. Este incremento de los ácaros después de aplicar lambda-cyhalotrina ha sido observado en otros estudios (Dively y Rose, 2002). En otros sistemas se ha demostrado la presencia de efectos ascendentes (“bottom-up”) en la abundancia de los depredadores cuando un recurso ubicado en un nivel trófico inferior aumenta (Badejo et al., 1995; Lövei y Sunderland, 1996). Si fenómenos de este tipo hubiesen estado presentes, éstos fueron largamente contrarrestados por la mortalidad causada por el insecticida, ya que el resultado neto final fue una disminución de la población de depredadores. Sin embargo, si el estudio hubiese estado diseñado en una escala temporal más amplia y hubiese estado más concentrado en las especies de menor tamaño (las que tendrían más probabilidades de depredar ácaros), tal vez se hubiese detectado algún tipo de efecto ascendente.

La asignación de las especies a grupos funcionales o a gremios no está libre de limitantes, ya que probablemente lo que se esté midiendo no sea el efecto del grupo funcional propiamente tal, si no que la capacidad del investigador de asignar correctamente las especies (Petchey y Gaston, 2002; Chalcraft y Resetarits, 2003). Bengtsson (1998) agrega que dependiendo del proceso ecológico de interés, los grupos funcionales pueden cambiar y por lo tanto especies pueden pertenecer a más de un grupo funcional dependiendo de los procesos ecológicos que estén en estudio.

A pesar de lo anterior, la adopción de este enfoque aún constituye una alternativa válida frente a las alternativas de estudiar las especies por separado (Gimmell, 2002; Sala et al., 1996), ya que permiten establecer al menos algunas relaciones mecanísticas que ayuden a

aumentar la capacidad de entender y predecir el funcionamiento del ecosistema y no limitarse a establecer sólo correlaciones (Bengtsson, 1998; Swift et al., 2004).

Respuesta de la comunidad de artrópodos como un todo.

Las comunidades de artrópodos presentes en los sitios de estudio fueron descritas en base a su diversidad, riqueza de especies, equitabilidad y dominancia. La comunidad fue dominada por taxa herbívoros, que representaron la mitad de las capturas, por detrítivoros y sólo en menor medida por depredadores y omnívoros, que juntos representaron menos de un 20% de las capturas.

En términos generales, la aplicación de las esporas no alteró ninguna de las propiedades de la comunidad, en las dos estaciones estudiadas. La aplicación de lambda-cyhalotrina tuvo efectos en cada una de ellas en las dos estaciones consideradas, con excepción de la riqueza de especies en el experimento de primavera. Por lo tanto, la comunidad de artrópodos fue menos diversa, tuvo menos especies y presentó una mayor dominancia como respuesta a la aplicación de este insecticida, con la excepción señalada anteriormente.

La relación entre la diversidad y el funcionamiento de los ecosistemas continúa siendo motivo de controversia entre los actores involucrados. A medida que la diversidad continúa declinando, más esfuerzos son dirigidos a establecer si esta reducción de diversidad puede afectar el funcionamiento, la estabilidad y la provisión de los llamados “servicios ecológicos”, entre ellos el control natural de plagas (Wilby y Thomas, 2002a; Romanuk et al., 2006). Numerosos investigadores adscriben a la idea que el funcionamiento de los ecosistemas depende en alguna medida de la diversidad (Wilby y Thomas, 2002b; Schwartz et al., 2000), pero esta posición no es unánime (Bengtsson, 1998). En el campo del control biológico, este debate también ha estado presente: Risch et al., (1983) revisaron más de 100 artículos publicados acerca de la relación entre diversidad y control de plagas. Este análisis los llevó a concluir que en el 53% de los casos había menos plagas en los sistemas más diversos, en el 18% había más plagas cuando aumentaba la diversidad, no había variación en el 8% de los casos y que en el 20% restante las respuestas eran erráticas.

Se ha propuesto que esta hipotética relación puede tomar tres diferentes formas: lineal (Ehrlich y Ehrlich, 1981), asintótica (Walker, 1992) o incluso idiosincrática (Lawton, 1994).

Cada una de estas formas propuestas refleja diferentes supuestos y dependiendo de estos supuestos, la reducción de la diversidad puede tener consecuencias más o menos importantes para el funcionamiento del ecosistema, su estabilidad y la provisión de "servicios ecológicos", entre ellos el control natural de plagas. Si la relación es lineal, entonces cualquier pérdida de diversidad, por mínima que sea, redundará en un menor desempeño del sistema. Si la relación es asintótica, entonces el sistema puede ser menos diverso y seguir funcionando, hasta sobrepasar un umbral a partir del cual el funcionamiento se ve afectado, mientras que si la relación es idiosincrática, las consecuencias de la pérdida de especies depende en gran medida de la identidad de las especies perdidas.

Siendo un debate aún abierto, es importante tener en cuenta estas consideraciones para poder asignar importancia a la reducción de la diversidad observada en este estudio. Sin embargo, el escaso conocimiento del funcionamiento de este agroecosistema, en especial de las relaciones tróficas presentes al interior de él, limita la capacidad de anticipar si la reducción de la diversidad de la artropofauna de la pradera afectará la regulación de los herbívoros. Las praderas actuales son el resultado de un proceso de deforestación y de pérdida de diversidad que ha durado cerca de 150 años. Por lo tanto, bajo el enfoque lineal, cualquier pérdida adicional de diversidad afectaría su funcionamiento.

En años recientes, la evidencia experimental reunida tiende a favorecer una relación de tipo asintótica entre la diversidad y el funcionamiento del ecosistema (Schwartz et al., 2000; Swift et al., 2004). Si adoptamos el enfoque asintótico, desconocemos en qué punto del proceso de pérdida de diversidad se encuentran las praderas y por ende una menor diversidad podría tener consecuencias leves (si la pradera estuviera en la parte asintótica de la curva) o podría tener consecuencias importantes (si estuviera en la parte exponencial de la curva o al inicio de la parte asintótica).

Bajo el tercer enfoque, resultaría aún más difícil anticipar las consecuencias de la pérdida de diversidad, ya que el estado actual del conocimiento de la pradera sólo permite sospechar cuáles especies tienen mayor influencia en el funcionamiento de ese sistema o si constituyen especies clave (“keystone species”).

Debido a las razones expuestas, considero que se debe adoptar un enfoque conservador ante la pérdida de diversidad en la pradera, ya que hasta que no decante el debate acerca de la relación entre diversidad y funcionamiento ecológico, incluyendo las diversas formas que esta relación podría tener, no se puede descartar que incluso pérdidas menores de diversidad podría traducirse en severos desequilibrios. Se debe considerar que en otros sistemas agrícolas bajo procesos de intensificación, la regulación de los artrópodos herbívoros fue afectada por la pérdida de diversidad mucho antes de que se identificara los mecanismos exactos por los cuales los herbívoros dejaron de ser regulados por sus depredadores (Hardin et al., 1995). Por lo tanto, esta evidencia señala que es deseable, pero no indispensable, conocer a cabalidad el funcionamiento de un agroecosistema para abogar por la conservación de sus componentes, incluida la diversidad, ya que cuando los sistemas agrícolas pierden diversidad generalmente tienden a desestabilizarse y a aumentar la frecuencia y magnitud de los brotes de plagas (Altieri, 1991; Swift et al., 1996).

A pesar de ser pobremente conocidos, se ha propuesto algunos mecanismos por los cuales la diversidad influiría en el funcionamiento de los agroecosistemas. Entre ellos (Wilby y Thomas, 2002b), cabe mencionar la complementariedad en el uso de los recursos (especies que ocupan nichos distintos permiten que esas especies funcionen complementariamente en ambientes heterogéneos o temporalmente variables) y otro mecanismo llamado efecto de muestreo (la probabilidad de incluir una especie influyente aumenta a medida que aumenta la diversidad). En la medida que se conozca cómo las especies se complementan unas con otras y hasta dónde ellas tienen un peso o influencia distintos en el proceso de interés, en este caso la regulación de los herbívoros, se podría aumentar la capacidad de predecir las consecuencias de la pérdida de diversidad.

En esta línea de razonamiento, las características de historia de vida del herbívoro influirían en la diversidad de sus enemigos naturales y en la forma cómo el control responde frente a reducciones de ella. Wilby y Thomas (2002a) plantean que los controladores de un herbívoro holometábolo, en este caso particular *D. pallens*, deberían tener más complementariedad entre sí comparados con los enemigos de un herbívoro hemimetábolo. Por lo tanto, simulaciones han mostrado que si disminuye la diversidad de los controladores de un herbívoro holometábolo, se produciría una inmediata aunque gradual reducción de su control. En contraste, el control de un herbívoro hemimetábolo no se resentiría por la pérdida de algunos de sus enemigos si no hasta que la pérdida de diversidad llegue a niveles extremos (Wilby y Thomas, 2002a). Siendo teóricas, estas consideraciones han sido consistentes con los patrones de emergencia de plagas en algunos sistemas agrícolas en proceso de intensificación (Wilby y Thomas, 2002b).

La diversidad es considera por algunos investigadores una propiedad abstracta y agregada que carece de relaciones directas con las funciones del ecosistema (Bengtsson, 1998; Chapin et al., 1996) y además entregan varias desventajas que limitan su aplicación. Sin embargo, incluso entre estas posiciones más críticas, se reconoce que mantener una alta diversidad es deseable para mantener un reservorio de especies que pueden realizar funciones a medida que cambian las necesidades humanas o las condiciones ambientales, aspecto que otros investigadores incluso han llegado a definir como una función más de la diversidad (seguro de capital natural o “natural insurance capital” *sensu* Folke et al., 1996). En apoyo a lo anterior, se debería considerar que si algunas especies fueran redundantes en cuanto a una función, no necesariamente tienen que ser redundantes cuando son evaluadas de acuerdo a otra función (Walker, 1991; Wellnitz y Poff, 2001).

Al asumir que especies que ocupan la misma posición trófica actúan de la misma forma, Chalcraft y Resetarits (2003) plantean que se está subestimando la importancia de las diferencias (variación) entre los depredadores. Por lo tanto, la pérdida de un depredador constituye la pérdida de un rol funcional único, lo que es particularmente relevante en sistemas donde existe control de tipo descendente (“top-down”). Puesto que no se ha

descartado la existencia de este tipo de efectos en las praderas en estudio, tampoco habría que descartar que la pérdida de algunos de los taxa afectados por lambda-cyhalotrina se traduzca en la irrupción de nuevas plagas.

Los estudios sobre diversidad tienden a concentrarse en la riqueza de especies, mientras que el otro componente de la diversidad, la equitabilidad, ha recibido mucha menos atención. Uno de los escasos estudios (Schwartz et al., 2000) que evalúa ambos factores concluyó que señalan que una alta equitabilidad es una de las condiciones necesarias para que los sistemas sean más estables cuando aumenta la diversidad.

La evaluación del riesgo de un agente de CB, en este caso particular *B. bassiana* aislamiento QU-B931, debería incluir cuatro factores (van Lenteren et al., 2006): identificación y caracterización del ACB; riesgos para la salud humana; eficacia y riesgos ambientales. A su vez, este último involucra estudiar el rango de huéspedes, el establecimiento, la dispersión y los efectos directos e indirectos en especies no plaga del agente de control.

Los efectos negativos fueron detectables sólo en algunos taxa depredadores pero se reflejaron a nivel de comunidad, confirmando que especies situadas más arriba en la cadena trófica son más propensas a la extinción que las situadas en la base, frente a cambios ambientales (Chalcraft y Resetarits, 2003; Duffy, 2003).

Swift et al (2004) señalan que algunas funciones del ecosistema pueden ser más resilientes que otras debido a que no todos los componentes de la comunidad tiene la misma probabilidad de perderse frente a una perturbación, en este caso particular la aplicación de biocidas para controlar un insecto. De acuerdo a los resultados obtenidos, la merma del tercer nivel trófico hace suponer que una de las primeras funciones ecológicas que se resentirían producto de la pérdida de diversidad sería la regulación de los insectos herbívoros o dicho en términos más agronómicos, el control natural de plagas. Esta suposición se cumpliría en la medida que los herbívoros presentes, o al menos algunos de ellos, sean efectivamente regulados por sus depredadores, ya que la depredación no es el único factor que regula las poblaciones de herbívoros, aunque sí es frecuente que ello

suceda en sistemas simples con pocos vínculos tróficos (Dyer y Stireman, 2003), los cuales son más propensos a experimentar fuertes cascadas tróficas descendentes que otros sistemas más diversos (Polis y Strong, 1996).

A pesar del énfasis que a menudo se coloca en identificar las interacciones más fuertes dentro de un ecosistema, ellas no son las únicas que influyen en el funcionamiento y estabilidad de las redes tróficas (Worm y Duffy, 2003). El valor de las interacciones débiles consistiría en amortiguar las oscilaciones entre recursos y consumidores y en disminuir la probabilidad de extinción (McCann et al., 1998), al menos en sistemas marinos (Neutel et al., 2002; Berlow, 1999). De acuerdo a lo anterior, tanto la composición (más ligada a las interacciones fuertes) y como la diversidad (más ligada a las interacciones débiles) influyen en la estructura, función y estabilidad de las comunidades, por lo tanto la pérdida de diversidad puede tener efectos negativos en las cadenas tróficas, independientemente de las especies involucradas (Worm y Duffy, 2003).

Futura investigación.

Varios investigadores han propuesto utilizar otros enfoques para evaluar los efectos no deseados del control de plagas, entre ellos el enfoque de los módulos comunitarios (Holt y Hochberg, 2001). Estos módulos se definen como un pequeño número de especies cuyas dinámicas e interacciones son tan fuertes que pueden ser comprendidas en forma aislada del resto de la comunidad (Hochberg et al., 1996; Holt, 1997). Desde un punto de vista práctico, es indispensable considerar el estado del conocimiento del sistema donde se desea aplicar este enfoque. Por ejemplo, para un módulo de tres especies, Mouquet et al., (2005) requirieron 15 años de datos y una veintena de parámetros para generar su modelo. Claramente, es necesario reunir más evidencia del funcionamiento de la artropofauna de las praderas antes de plantear hipótesis testeables por medio de los módulos, profundizando estudios de exclusión y de análisis de contenido estomacal como los realizados en la última década en el sur de nuestro país (Alarcón, 1997; Morales, 2000; Espíndola, 2004).

La identificación de los servicios ecológicos y su posterior difusión más allá de los círculos especializados ciertamente contribuirá a una mayor valoración y protección de los

organismos que los proveen (Mooney, 2002; Kranz, 2000). En el caso estudiado, muy pocas de las especies de artrópodos involucrados son especies carismáticas o que por sí mismas atraigan la atención del público general. En la medida que se devele el rol de cada una de ellas en las praderas y se clarifique su importancia relativa, sería deseable adoptar el consejo entregado por estos autores y difundir estos hallazgos primero entre los actores más directamente involucrados (agricultores) y luego hacia otros sectores de la sociedad. Tal vez ningún otro tópico ecológico haya atraído tanto la atención del público general como aquellos relacionados con la biodiversidad. Esto ha desafiado las formas tradicionales de relacionarse entre los científicos y el resto de la sociedad, creando una oportunidad para re-pensar y re-crear la forma en que, manteniendo la integridad del método científico como esencia de su ser, la comunidad de investigadores logra establecer una comunicación efectiva con el resto de la sociedad (Mooney, 2002).

El control biológico basado en hongos es una interacción entre especies extremadamente compleja y abarca aspectos tan importantes como la transmisión horizontal, cambios conductuales (mayor propensión a la depredación, menor consumo) y otros efectos sub-letales causados por hongos como *B. bassiana* y otros (Thomas, 1999), todos los cuales ameritan ser incluidos dentro de la adecuada evaluación de sus efectos.

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ANEXOS

Anexo 1. Índices de severidad de los efectos no deseados del CB sugeridos por Lynch y Thomas (2000).

Severidad	Tipo de impacto
0	Sin registros de consumo, infección, parasitismo, supresión de la población o extinción.
1	-5% de mortalidad inducida por consumo/infección/ parasitismo o efectos sub-letales en fecundidad, sin registro de consecuencias significativas para la población.
2	5-40% de mortalidad inducida por consumo/infección/ parasitismo, sin registro de consecuencias significativas para la población.
3	+40% de mortalidad inducida por consumo/infección/ parasitismo (en una ocasión en una población local) y/o efectos sub-letales en fecundidad, sin registro de consecuencias significativas para la población.
4	+40% reducción de corto plazo de una población local o reducción permanente significativa (+10%) de una población local.
5	+40% reducción de largo plazo de una población local o +10% de una reducción de largo plazo de una población que cubra un área grande (100 x 100 km o más)
6	+40 de reducción de largo plazo de una población que cubra un área grande (100 x 100 km o más).
7	Aparente extinción de una población que cubra un área pequeña, donde la recolonización parezca probable en el largo plazo.
8	Extinción certificada en un área pequeña, donde la recolonización sea improbable o imposible.
9	Extinción certificada de una población en un área de 100 x 100 km o más.

Anexo 2. Categorías para la evaluación de riesgo basadas en el efecto total de los pesticidas (adaptado de Amano y Haseeb 2001).

Categoría	% de mortalidad o de reducción de la capacidad benéfica.
Laboratorio	
No dañino	-30%
Levemente dañino	30-79
Moderadamente dañino	80-99
Severamente dañino	+99
Semi-campo (actividad persistente)	
Vida corta	- 5 días
Levemente persistente	5-15 días
Moderadamente persistente	16-30 días
Persistente	+30 días
Semi-campo y campo	
No dañino	- 25
Levemente dañino	25-50
Moderadamente dañino	51-75
Severamente dañino	+75

Anexo 3. Capturas provenientes de los cilindros de suelo antes de la aplicación de los tratamientos, experimento de invierno (Osorno, julio de 2003).

		Control	*B.bassiana* B931	Lambda-cyhalothrin
ARACHNIDA				
OROBATIDA		172	244	201
LYCOSIDAE		4	2	4
GNAPHOSIDAE		18	43	21
COLEOPTERA				
CARABIDAE	*Argutoridius chilensis*	9	13	6
	Carabidae (n/i larvae)	2	1	1
CURCULIONIDAE	*Apion* sp.	0	1	0
	Listronotus bonariensis	10	46	36
	Other Curculionidae	1	8	6
	Curculionidae (n/i larvae)	0	1	0
SCARABAEIDAE	*Hylamorpha elegans*	1	0	1
STAPHYLINIDAE		5	4	8
CANTHARIDAE		159	696	455
ELATERIDAE		8	11	39
OTHER COLEOPTERA		17	26	3
LEPIDOPTERA				
HEPIALIDAE	*Dalaca pallens*	10	7	6
NOCTUIDAE		12	15	4
OTHER LEPIDOPTERA		0	13	5
DIPTERA				
ASILIDAE		4	10	1
STRATIOMYIDAE		12	8	3
TIPULIDAE		6	6	0
OTHER DIPTERA		9	19	6
ORTHOPTERA		1	6	1
DERMAPTERA				
FORFICULIDAE	*Forficula auricularia*	0	0	1
HEMIPTERA				
NABIDAE		0	0	1
OTHER HEMIPTERA		2	1	0
DICTYOPTERA				
MANTIDAE		0	3	1
CHILOPODA		4	15	16
OLIGOCHAETA				
LUMBRICIDAE		29	11	16
TOTAL		495	1210	842

Anexo 4. Capturas provenientes de los cilindros de suelo 20 días después de la aplicación de los tratamientos, experimento de invierno (Osorno, agosto de 2003).

		Control	*B.bassiana* B931	Lambda-cyhalothrin
ARACHNIDA				
OROBATIDA		197	195	196
LYCOSIDAE		1	8	2
GNAPHOSIDAE		26	43	3
COLEOPTERA				
CARABIDAE	*Argutoridius chilensis*	18	17	4
	Ferionomorpha sp.	1	0	0
	Carabidae (n/i larvae)	3	0	1
CURCULIONIDAE	*Apion* sp.	2	2	0
	Listronotus bonariensis	38	104	18
	Other Curculionidae	3	8	0
	Curculionidae (n/i larvae)	2	0	0
SCARABAEIDAE	*Hylamorpha elegans*	1	1	0
STAPHYLINIDAE		3	23	1
CANTHARIDAE		158	436	449
ELATERIDAE		4	9	20
OTHER COLEOPTERA		6	35	0
LEPIDOPTERA				
HEPIALIDAE	*Dalaca pallens*	5	2	0
NOCTUIDAE		4	7	0
OTHER LEPIDOPTERA		3	43	2
DIPTERA				
ASILIDAE		2	11	2
STRATIOMYIDAE		3	0	0
TIPULIDAE		9	4	1
ORTHOPTERA		0	1	0
HEMIPTERA				
NABIDAE		0	2	0
OTHER HEMIPTERA		1	6	0
CHILOPODA		0	7	5
OLIGOCHAETA				
LUMBRICIDAE		15	5	7
TOTAL		505	969	711

Anexo 5. Capturas provenientes de los cilindros de suelo 40 días después de la aplicación de los tratamientos, experimento de invierno (Osorno, septiembre de 2003).

		Control	*B.bassiana* B931	Lambda-cyhalothrin
ARACHNIDA				
OROBATIDA		246	332	330
LYCOSIDAE		11	15	1
GNAPHOSIDAE		23	43	3
COLEOPTERA				
CARABIDAE	*Argutoridius chilensis*	15	14	13
	Ferionomorpha sp.	0	1	2
	Metius flavipes	2	3	0
	Carabidae (n/i larvae)	7	9	0
OTHER CARABIDAE		1	1	0
CURCULIONIDAE	*Apion* sp.	1	9	0
	Listronotus bonariensis	37	130	20
	Other Curculionidae	3	6	1
	Curculionidae (n/i larvae)	3	2	0
SCARABAEIDAE	*Hylamorpha elegans*	1	0	1
STAPHYLINIDAE		10	12	9
CANTHARIDAE		179	402	299
ELATERIDAE		2	1	0
	N/I larvae	10	10	22
OTHER COLEOPTERA		14	33	0
LEPIDOPTERA				
HEPIALIDAE	*Dalaca pallens*	2	1	0
NOCTUIDAE		1	5	0
PYRALIDAE		1	0	2
OTHER LEPIDOPTERA		6	40	0
DIPTERA				
ASILIDAE		7	5	0
STRATIOMYIDAE		3	0	0
TIPULIDAE		5	2	0
DERMAPTERA				
FORFICULIDAE	*Forficula auricularia*	1	0	0
CHILOPODA		3	5	13
OLIGOCHAETA				
LUMBRICIDAE		20	13	17
TOTAL		614	1094	733

Anexo 6. Capturas provenientes de los cilindros de suelo antes de la aplicación de los tratamientos, experimento de primavera (Valdivia, 15 de octubre de 2003).

		Control	*B.bassiana* B931	Lambda-cyhalothrin
ARACHNIDA				
OROBATIDA		114	122	138
LYCOSIDAE		1	0	0
GNAPHOSIDAE		22	33	40
COLEOPTERA				
CARABIDAE		32	30	46
	Carabidae (n/i larvae)	70	65	76
CURCULIONIDAE	*Apion* sp.	38	38	37
	Listronotus bonariensis	20	13	19
	Other Curculionidae	3	4	7
	Curculionidae (n/i larvae)	7	6	7
STAPHYLINIDAE		20	12	14
CANTHARIDAE		5	10	6
ELATERIDAE		1	0	1
	N/I larvae	3	0	3
APHODIDAE		18	14	22
OTHER COLEOPTERA		7	12	3
LEPIDOPTERA				
HEPIALIDAE	*Dalaca pallens*	1	0	0
NOCTUIDAE		2	1	0
OTHER LEPIDOPTERA		2	4	4
DIPTERA				
ASILIDAE		0	0	1
STRATIOMYIDAE		1	0	2
TIPULIDAE		0	0	2
CHILOPODA		0	1	0
OLIGOCHAETA				
LUMBRICIDAE		37	59	43
TOTAL		404	424	471

Anexo 7. Capturas provenientes de los cilindros de suelo antes de la aplicación de los tratamientos, experimento de primavera (Valdivia, 15 de noviembre de 2003).

		Control	*B.bassiana* B931	Lambda-cyhalothrin
ARACHNIDA				
OROBATIDA		108	123	151
LYCOSIDAE		1	1	0
GNAPHOSIDAE		21	16	7
COLEOPTERA				
CARABIDAE		16	11	1
	Carabidae (n/i larvae)	72	50	40
CURCULIONIDAE	*Apion* sp.	11	11	8
	Listronotus bonariensis	17	16	19
	Curculionidae (n/i larvae)	32	17	10
STAPHYLINIDAE		51	31	18
CANTHARIDAE		1	0	2
ELATERIDAE	N/I larvae	0	3	0
APHODIDAE		9	1	4
OTHER COLEOPTERA		6	12	8
LEPIDOPTERA				
HEPIALIDAE	*Dalaca pallens*	1	0	0
NOCTUIDAE		0	1	0
OTHER LEPIDOPTERA		33	38	6
DIPTERA				
DERMAPTERA				
FORFICULIDAE	*Forficula auricularia*	0	3	1
ASILIDAE		1	0	0
STRATIOMYIDAE		0	0	2
TIPULIDAE		0	3	0
DICTYOPTERA				
MANTIDAE		2	1	0
LUMBRICIDAE		27	32	27
TOTAL		409	370	304