

Integration and impacts of invasive plants on plant-pollination networks

Integración e impactos de las plantas invasoras sobre las redes de plantas y polinizadores



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***Integration and impacts of invasive plants
on plant-pollination networks***

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“Oh sol que alivias mi visión turbada!
Me alegras tanto al resolver mis dudas
que, tanto cual saber, dudar me agrada”

-Dante Alighieri-

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Esta tesis ha resultado ser invasora, y como buena invasora, su integración en el ecosistema de mi vida ha sido muy bueno, estableciendo interacciones (más o menos fuertes) con todas las especies que circundan mi comunidad. La red de interacciones se empezó a tejer en el CREAM, gracias a Montse Vilà, que a lo largo de estos años siempre ha sido mi *referee* más tenaz. La tesis enseguida invadió el despacho, llenándolo de artículos, bichos y preguntas y de repente también de risas y música, y lo convirtió en el despacho más molón del reino (you know, el despacho somos todos). Invadió el Cap de Creus, las playas, los montes y las horas al sol. Invadió media Alemania y se puso a escalar en Frankenjura. La tesis se fue extendiendo y se coló por los resquicios de las puertas del CREAM y hasta llegó a instalarse a tiempo parcial en el despacho de Jordi Bosch, pero la presión de propágulo era tan intensa que se naturalizó también en casa*. La tesis invadió conversaciones con *estos* y *estos* invadieron a su vez un poco la tesis. Se incorporó a cientos de conversaciones con Belén y finalmente hasta fue adoptada por mis padres y se metía en mi mochila cada vez que escalábamos por el Montsec. Y eso por no hablar de los miles de Kb que ha transferido por internet. Ni, por supuesto, de Lo Vuestro. La comunidad receptora seguro que ha sufrido sus impactos, pero las comunidades son muy resilientes y los caminos de la coevolución inescrutables, de manera que estoy contento de que estos cuatro años no sólo me hayan *enriquecido* a mí. La ciencia se construye entre todos. Todos los que alguna vez habéis hecho un experimento, todos los que habéis puesto en duda algo, todos los que os habéis preguntado acerca de esa cosa tan rara. Gracias a todos.

*Casa pa's, cerdanyola, el niu o el newniu, y ahora más que nunca el Nda.

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Introducción general

Resumen

Las especies invasoras son aquellas que transportadas e introducidas por el ser humano en lugares fuera de su área de distribución natural, han conseguido establecerse y dispersarse en la región de destino. Las especies invasoras pueden producir cambios en las poblaciones de las especies nativas e incluso conducir a su extinción local. Las comunidades invadidas por plantas exóticas pueden sufrir diversos impactos, siendo la competencia por los recursos ambientales, los cambios en las propiedades del suelo o en el régimen de perturbaciones los más estudiados. Sin embargo, los efectos sobre otros niveles tróficos han recibido menos atención, aunque cada vez hay más estudios referentes al efecto sobre los herbívoros y los mutualismos. La mayoría de estudios sobre el impacto de las plantas invasoras en las interacciones de polinización han considerado interacciones entre pares de especies. Dado que las plantas suelen interactuar con varios polinizadores y los polinizadores visitar varias especies de plantas, las especies invasoras tienen el potencial de perturbar, no sólo interacciones puntuales, sino toda la red de interacciones. No obstante, se ha realizado poca investigación sobre las repercusiones de las invasiones a nivel de toda la comunidad de polinizadores y plantas. Esta tesis estudia la forma en que los sistemas de plantas y polinizadores se ven afectados por especies de plantas invasoras. En este primer capítulo, presento la tesis en un contexto general, considerando las plantas invasoras en el marco del cambio global, los mutualismos según el enfoque de la estructura de redes y lo que se conoce hasta la fecha acerca de los efectos de las plantas invasoras en los sistemas de polinización.

Las plantas invasoras y el cambio global

Como consecuencia del aumento de la población humana, del transporte, y de la explotación de los recursos naturales (diCastrì 1989; Constanza y otros 1997; Daily 1997; Goudie 2000), la diversidad biológica, en todas sus distintas escalas, está disminuyendo a un ritmo sin precedentes (Pimm y otros 1995; Sala y otros 2000). Históricamente, el principal impulsor de los cambios ambientales en los ecosistemas terrestres ha sido la destrucción del hábitat, principalmente a través de la transformación de los hábitats naturales en sistemas agrícolas y urbanos. Junto con la



pérdida de hábitat, el cambio climático, la sobreexplotación, la contaminación y las invasiones biológicas son otros componentes de cambio global muy relevantes que en muchas circunstancias actúan sinérgicamente (Walker y Steffen 1997; Sala y otros 2000), aun así, la interacción entre distintos componentes del cambio global se ha explorado poco. Las invasiones biológicas implican una redistribución de las especies a escala planetaria (Elton 1958; Kowarik 1990; Jenkins 1996; Pyšek 1998; Hobbs 2000) y han pasado a considerarse como una de las mayores amenazas para la diversidad biológica (Lodge 1993; Vitousek y otros 1997; Wilcove y otros 1998).

Las especies invasoras son aquellas que transportadas e introducidas por el ser humano en lugares fuera de su área de distribución natural, han conseguido establecerse y dispersarse en la región de destino (Richardson y Pyšek 2000). En los últimos 500 años, la introducción deliberada y/o accidental supera en su alcance, frecuencia e impacto al movimiento de organismos por fuerzas naturales en toda la duración del período de la historia de la tierra (Mack y otros 2000). El transporte puede ser intencional, causado por el hombre con un fin determinado, como la producción de alimentos, madera, mejora del suelo o jardinería; o accidental, de forma involuntaria y asociada a las rutas de comunicación, cargamentos de productos agrícolas o el abatimiento de barreras geográficas por obras de ingeniería. Para el caso de especies vegetales, la mayoría de especies no superan la fase de transporte e implantación, pero aún así, son muchas las que prosperan (Di Castri 1989; Williamson 1996). Una parte de estas especies introducidas se naturalizan, llegando a formar poblaciones autosostenibles (Kowarik 2003); de estas, algunas poseen una tasa de expansión muy rápida pasando a considerarse invasoras (Richardson y Pyšek 2000).

Las plantas invasoras no han evolucionado en la comunidad receptora, sin embargo, son capaces de establecerse con mucho éxito e incluso desplazar especies nativas que han tenido mucho más tiempo para adaptarse al ambiente. Este éxito depende de características propias de la especie tales como preadaptación a las nuevas condiciones climáticas, sistema reproductivo flexible, etc; de la presión de propágulos, es decir, la abundancia y frecuencia de la introducción; y de la vulnerabilidad del ecosistema a la invasión o invasibilidad (Kühn y otros 2003, Rejmánek y otros 2005, Pyšek y Jarosík 2005, Hamilton y otros 2005), el cual depende en gran medida de la presencia de nichos ecológicos vacíos, la falta de enemigos naturales, y la facilidad para establecer



relaciones mutualistas (Lonsdale 1999). El hecho de que haya tantos factores involucrados en el proceso de invasión, explica que no todas las especies exóticas acaben siendo invasoras, ni que todos los ecosistemas acaben siendo invadidos en igual grado.

Las especies invasoras pueden causar impactos ecológicos y económicos de diferente magnitud. En el ámbito ecológico el principal impacto de las invasiones biológicas es la pérdida de biodiversidad y la homogeneización de los sistemas biológicos (Lockwood y McKinney 2001). La introducción de una especie exótica puede alterar la abundancia de las especies e incluso causar la extinción local (Atkinson y Cameron 1993, Lodge 1993). El impacto en las especies nativas se produce por competencia, depredación, herbivoría, producción de sustancias tóxicas, hibridación con especies nativas emparentadas, modificación de las propiedades de los ecosistemas y/o modificación del régimen de perturbaciones (Vitousek y otros 1987, D' Antonio y Vitousek 1992, Vitousek 1994, D' Antonio y Corbin 2003, Levine y otros 2003). Respecto a los impactos económicos las especies exóticas son responsables de pérdidas debidas a daños en la producción de las cosechas, la ganadería y en las infraestructuras. Solo en los Estados Unidos los daños económicos asociados a las invasiones biológicas ascienden a 137 Billones de dólares anuales (Pimentel y otros 2001). Por otra parte, también ocasionan costes derivados de su control y/o erradicación. En España la inversión en el control de plantas invasoras en espacios naturales asciende a más de 50 millones de euros en los últimos 10 años (Andreu y Vilà 2007).

Las interacciones mutualistas: la polinización

Las interacciones entre organismos desempeñan un papel clave en la estructura de las comunidades ecológicas (Thomson 1996), determinan las características de los ecosistemas (Chapin y otros 2000) y directa o indirectamente afectan sus procesos (Chapin y otros. 2000; Hooper y otros. 2005). Los resultados ecológicos y evolutivos generados por las interacciones varían geográficamente, por lo tanto, magnifican la biodiversidad y el número total de las especies, siendo su preservación fundamental para el mantenimiento de la diversidad de especies en sí (Thompson 1996). El mutualismo es una interacción en la que los organismos implicados obtienen



algún grado de beneficio. Las interacciones mutualistas condicionan procesos ecológicos tan importantes como pueden ser la polinización de las plantas y la dispersión de semillas.

La polinización de las plantas mediada por animales es un mutualismo que desempeña un papel central en la reproducción y en el establecimiento tanto de las poblaciones de las especies de plantas, como de los animales que intervienen (Burd 1994; Ghazoul 2002; Jordano y otros 2003). Más del 90% de las angiospermas son polinizadas por animales (Buchmann y Nabhan 1996) y entre un 60 a 70% de las especies de plantas dependen de la polinización por insectos (Richards 1997). Asimismo, los polinizadores prestan un servicio para los seres humanos al ser responsables de la producción de frutas y semillas de muchas plantas de cultivo (Kearns y otros 1998). Por ejemplo, en Estados Unidos se ha estimado que el valor anual de las abejas para la polinización de cultivos es de 14.6 millones de dólares anuales (Morse y Calderone 2000, Losey y Vaughan 2006) y que los insectos polinizadores nativos pueden ser responsables de polinizar frutas y hortalizas producidas por valor de 3.07 millones de dólares.

En los últimos años, la conservación de las interacciones planta-polinizador ha sido de gran preocupación (Allen-Wardell y otros 1998; Kearns y otros 1998; Kevan 1999; Kremen y Ricketts 2000; Cane y Tepedino 2001; Kevan y Phillips 2001), en respuesta a la disminución de las poblaciones de polinizadores (Kearns y otros 1998; Ghazoul 2005, Beisjmier y otros 2006). La introducción de especies exóticas se considera una de las amenazas a las que se enfrentan los sistemas de polinización (Kearns y otros 1998; Kevan 1999), pero todavía hay poca comprensión sobre la forma en que las especies invasoras afectan en estos sistemas.

Las redes planta-polinizador

Las primeras clasificaciones de los sistemas de polinización que llevaron a cabo Sprengel (1793), Delpino (1868-1875) y Knuth (1906, 1908), entre otros, condujeron a la clasificación de las morfologías florales en síndromes de polinización (Vogel 1954, Van der Pijl 1961, Faegri y van der Pijl 1966, Baker y Hurd 1968). Estos asumen que las flores se han especializado en especies de



“polinizadores tipo”, convergiendo en diferentes síndromes (Fenster y otros. 2004, Waser 2006). Este punto de vista prevaleció hasta hace poco, pero cada vez es más reconocido que la mayoría de las especies con flores están visitadas por una gama de visitantes taxonómicamente muy diversa, y por tanto, los sistemas de polinización tienden a ser generalistas (Herrera 1988; Herrera 1996; Ollerton 1996; Waser y otros 1996; Waser 1998).

Por lo general, las interacciones mutualistas involucran a decenas e incluso centenares de especies que forman redes complejas de interdependencia. Entender cómo responde la biodiversidad y los servicios que reportan ante las crecientes perturbaciones de origen antrópico requiere una aproximación a nivel de comunidad, ya que desde un punto de vista coevolutivo, tan importante es conocer qué especies integran la comunidad, como cuáles son las dependencias entre especies (Thomson 2001).

Estudios recientes han descrito las principales propiedades y características de las redes mutualistas. El primer trabajo que aplicó trato las interacciones de plantas y polinizadores como redes tróficas fue llevado a cabo por Jordano (1987). Este estudio explora la especificidad y generalismo de los diferentes grupos de polinizadores e indaga en las pautas de conectancia (interacciones reales que ocurren dentro de todas las posibles). Sin embargo, fue Memmott (1999) quien caracterizó por primera vez toda una comunidad de plantas y polinizadores con un enfoque de red bimodal (Figura 1.1). Jordano y otros (2003) también han estudiado la distribución de conectividades (número de interacciones por especie) y han llegando a la conclusión de que es muy heterogénea y las especies están mucho más conectadas de lo esperado por azar. Dicha distribución es una descripción estadística que nos predice un número de interacciones, pero no nos explica cuál es el patrón de relación entre plantas y polinizadores. Por tanto, el estudio de la distribución de conectividades constituye un primer paso en la caracterización del grado de estructura de las redes mutualistas al que hay que añadir el patrón de anidamiento. Bascompte y otros (2003) estudiaron una propiedad estructural de la red de interacciones: su grado de anidamiento. Una red es anidada si una especie de planta interactúa con un subconjunto específico de los animales con los que interactúa una especie de planta más generalista (Figura 1.2). La mayoría de las redes mutualistas son anidadas. Además se ha demostrado que las interacciones planta-polinizador tienden a ser asimétricas (Vázquez y Aizen 2004), con especies



generalistas interactuando principalmente con especies especialistas. Existe una fuerte correlación entre la abundancia y la generalización de las especies (Vázquez y Aizen 2003; Vázquez y Aizen 2006), es decir, las especies generalistas son más abundantes mientras que las especializadas tienden a ser raras. Las redes planta-polinizador son muy cohesivas, a diferencia a otro tipo de organización, como por ejemplo la compartimentación en grupos de especies encontrados en las redes tróficas clásicas de presa-depredador.

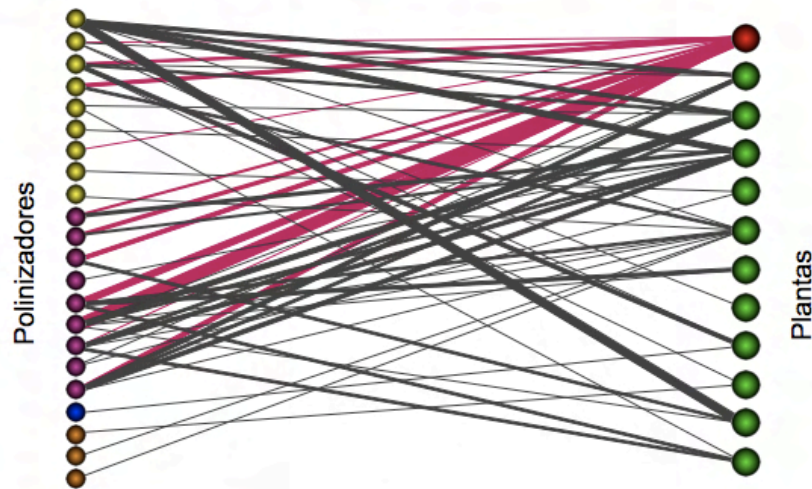


Figura 1.1: Representación de una red invadida por *Carpobrotus aff. acinaciformis* (círculo rojo). A la derecha se indican las especies de plantas (verde) y a la izquierda los diferentes órdenes de polinizadores: Himenópteros (amarillo), coleópteros (rosa), dípteros (azul) y lepidópteros (naranja). El grosor de las interacciones es proporcional a su frecuencia

El último gran avance en la comprensión de la estructura de las redes mutualistas trata no sólo de qué interacciones se dan (redes cualitativas), sino en qué frecuencia ocurre cada interacción (redes cuantitativas). Bascompte y otros (2006), analizando con qué frecuencia se produce cada tipo de interacción, describieron cómo el patrón de interacciones se caracteriza por el dominio de dependencias muy débiles, es decir, las plantas dependen poco para su reproducción de una sola especie determinada de polinizador y viceversa, y asimétricas (en las pocas ocasiones en las que una especie depende mucho de otra, esta apenas depende de la primera, ya que interactúa con muchas otras especies). Así, estas redes de interacciones de beneficio mutuo débiles y asimétricas fomentan la coexistencia de un gran número de especies. Comprender la estructura de estas redes es fundamental para entender el proceso coevolutivo y la estabilidad de dichas comunidades, aspectos que no pueden alcanzarse reduciendo las comunidades a pares de especies aislados.



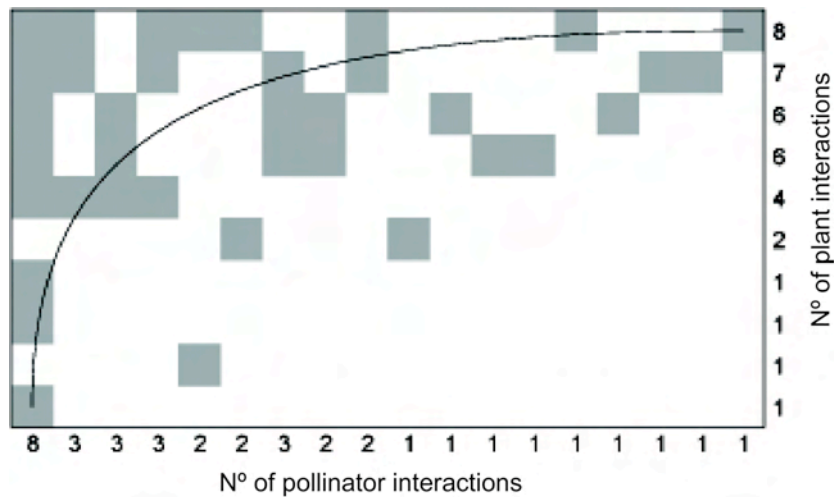


Figure 1.2: Ejemplo de una estructura anidada en una red no invadida por *Carpobrotus aff. acinaciformis*. Los números en las filas representan el número de interacciones de cada planta y en las columnas de cada polinizador. Los cuadrados llenos indican una interacción entre planta y polinizador. También se muestra la isoclina de anidamiento perfecto (Nestness = 0.83).

Las plantas invasoras como nuevo elemento en las redes planta-polinizador

La introducción de una especie y sus mutualistas (ej, polinizadores, dispersores) o antagonistas (ej, predadores, parásitos, competidores) raramente ocurre de forma simultánea. Por tanto, en la comunidad de introducción, una especie invasora debe establecer nuevas interacciones. Las nuevas interacciones mutualistas pueden incluso facilitar la invasión, creando nuevas oportunidades tróficas y así promoviendo su integración en las comunidades receptoras (Richardson y otros 2000). De hecho, diversos estudios han mostrado ya que las interacciones mutualistas tienen una importancia clave en determinar el éxito de plantas invasoras, sobre todo aquellas que no son auto-compatibles (Parker y Haubensak 2002). A la vez, las especies invasoras pueden alterar dramáticamente las interacciones mutualistas de la comunidad invadida (Traveset y Richardson 2006), las cuales a su vez pueden volver a influir sobre la dinámica de la invasión (Mitchell y otros 2006, Didham y otros 2005). Por tanto, hay que tener en cuenta la bidireccionalidad de los efectos entre las invasiones biológicas y las interacciones mutualistas.



La presencia de una especie invasora puede afectar a la polinización de una planta nativa por medio de cambios tanto en el comportamiento (i.e. alteración de los patrones de visita) como en la abundancia de los polinizadores (i.e. incrementos o disminución en sus poblaciones), aunque todos los estudios que han evaluado cambios en la polinización de una especie nativa frente a la presencia de una invasora se han focalizado en el primero de estos mecanismos (Bjerknes 2007; Figura 1.3). La mayoría de estudios se han limitado a analizar sus impactos sobre las visitas y el cuajado de semillas de especies de plantas nativas focales (Grabas y Laverty 1999; Brown y Mitchell 2001; Chittka y Schurkens 2001; Brown y otros. 2002; Ghazoul 2002; Aigner 2004; Moragues y Traveset 2005) y se han hipotetizado los mecanismos por los que las plantas invasoras pueden estar afectando a la polinización de las plantas nativas (Traveset y Richardson 2006). En primer lugar, pueden alterar los patrones de visita de los insectos a las especies nativas (Ghazoul 2002), y esto puede afectar a la calidad y la cantidad de polen depositado en los estigmas. Esto tiene el potencial de reducir (o aumentar) el cuajado y la calidad de semillas, y a la larga modificar la estructura genética de las poblaciones de especies afectadas (Traveset y Richardson 2006). Además, si las especies nativas e invasoras están relacionadas taxonómicamente, la deposición de polen interespecífico en estigmas podría dar lugar a procesos de hibridación, y afectar al vigor tanto de las especies nativas como invasoras (Brown y otros 2002). Por tanto, el “usurpo de polinizadores”, o competencia por los mismos, ha sido hasta la fecha el mecanismo más estudiado, bajo la hipótesis de que una especie invasora más atractiva o que ofrece más recursos a los polinizadores atrae muchos polinizadores, y la especie nativa sufre una reducción en la cantidad de visitas, con la consecuente disminución en los niveles de la polinización y posterior reducción en la producción de semillas. Sin embargo, sólo 2 de los 7 estudios que hay publicados encuentran una reducción en el cuajado de semillas (Chittka y Schurkens 2001, Brown y Mitchell 2001). Otros estudios han encontrado una reducción en el número de visitas (Totland y otros 2006) pero no se ve efecto en el cuajado de semillas. Alternativamente, la “atracción conjunta” de polinizadores por parte de especies nativas y invasoras puede dar lugar a una interacción positiva o “facilitación” en la que la presencia de la planta invasora incrementa las visitas a las nativas al atraer una mayor diversidad y/o abundancia de polinizadores). De hecho, existen razones teóricas para esperar que las interacciones positivas sean igualmente probables que las negativas ya que muchas plantas invasoras ofrecen gran cantidad de recursos capaces de movilizar gran cantidad de polinizadores, actuando como “especies magnéticas” para ciertos grupos de polinizadores (Lopezaraiza-Mikel y otros 2007). La polinización en circunstancias naturales es muy variable en el espacio y el tiempo (Schemske y Horvitz 1984; Herrera 1988; Thomson 2001) y depende de muchas variables



ecológicas. Por lo tanto no es sorprendente que los efectos encontrados sean tanto positivos como negativos (Larson y otros 2006).

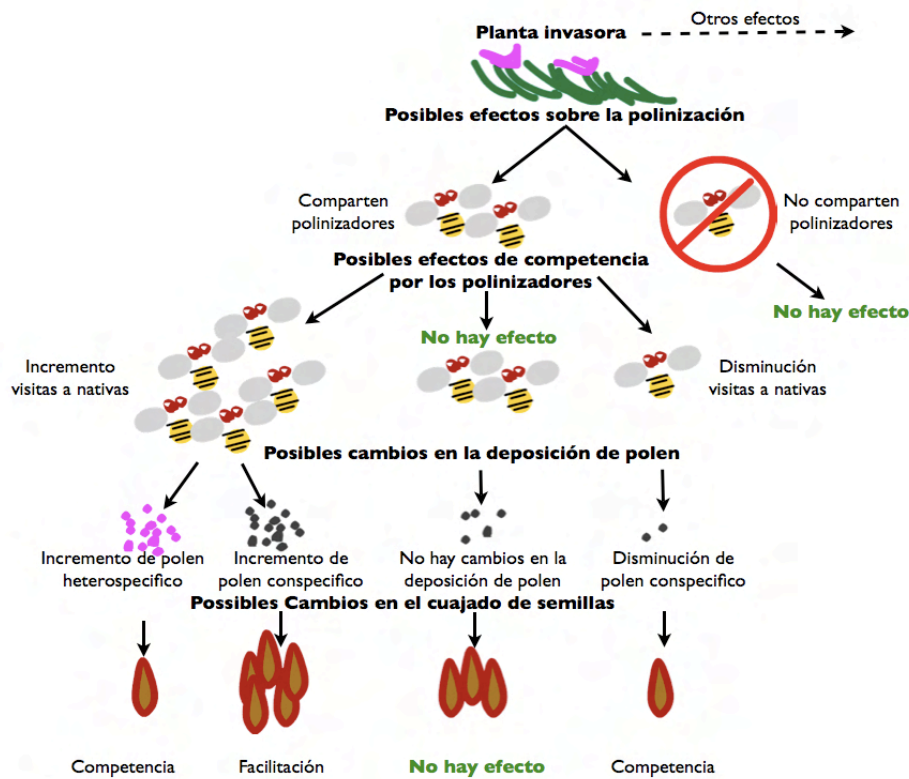


Figura 1.3: Marco conceptual simplificado de los posibles mecanismos por los cuales las plantas invasoras pueden afectar a la polinización de las plantas nativas (inspirado en Bjerknes y otros 2007).

Hay que tener en cuenta que, además de la cantidad de visitas a las flores, la presencia de una especie invasora puede llevar a una reducción en la calidad de estas visitas, por ejemplo, si el polinizador realiza visitas entre flores nativas e invasoras, y el polen heteroespecífico interfiere con la deposición y/o germinación del polen propio de la especie (Jackobson et al. 2008). Estos cambios pueden ocurrir independientemente de cambios en la frecuencia absoluta de visitas, aunque en algunos casos ambos fenómenos ocurren simultáneamente (Lopezaraiza-Mikel y otros 2007). Por tanto es importante investigar no sólo el patrón de visitas, sino toda la cadena de transporte de polen desde las anteras de la planta invasora hasta los estigmas de las plantas nativas.

En contrapartida a la atención que ha recibido la competencia por los polinizadores con las plantas nativas, el efecto de las plantas invasoras sobre las abundancias de polinizadores ha recibido



relativamente poca atención y se ha abordado indirectamente al estudiar el éxito de la reproducción de las plantas invasoras (Parker 1997, Parker y Haubensak 2002; Stout y otros 2006). Las plantas invasoras pueden afectar el comportamiento de los polinizadores nativos al establecer interacciones con ellos que modifiquen sus poblaciones (Traveset y Richardson 2006), ya sea positivamente facilitando su mantenimiento (Graves y Shapiro 2003) o negativamente alterando las relaciones de competencia entre diversos grupos. Los efectos indirectos derivados del desplazamiento de las plantas nativas sobre los polinizadores también son muy desconocidos.

Los estudios detallados sobre los efectos de especies invasoras sobre especies nativas han sido importantes para revelar la diversidad de formas en que las especies nativas pueden ser afectadas. Sin embargo, el estudio a nivel de comunidad, aportan una visión de conjunto que permite una comprensión mejor del impacto global de las plantas invasoras. Primero, definimos integración de una planta invasora en una comunidad como la capacidad de la especie invasora de incorporarse a la red nativa de interacciones planta-polinizador, es decir, de establecer enlaces con las especies residentes. Por tanto, Entre los atributos de las especies que permiten predecir su éxito invasor y su integración en las redes mutualistas está la amplitud de nicho ecológico (Vázquez 2005). Se ha predicho que especies exóticas generalistas tendrán mayores posibilidades de recibir visitas que especies especialistas (Richardson y otros 2000). Sería entonces esperable que las especies invasoras fueran más generalistas que las especies nativas, ya que durante el proceso de establecimiento, una fracción de las exóticas especialistas hubieran fracasado. Sin embargo, comparaciones del grado de generalización de especies nativas con el de las invasoras no han corroborado esta hipótesis (Vázquez 2005, Memmott y Waser 2002, Olesen y otros 2002).

En cambio, estudios en comunidades naturales invadidas han demostrado que el grado de generalización de los polinizadores nativos sí se correlaciona positivamente con la probabilidad de incluir una determinada planta invasora (Lopezaraiza-Mikel y otros 2007) o con el número de plantas invasoras incluidas en su dieta (Memmott y Waser 2002). De hecho, la mayoría de las plantas invasoras más exitosas son visitadas por polinizadores generalistas (Richardson 2002, Lopezaraiza-mikel y otros 2007). Dado que los sistemas de polinización tienden a ser generalistas, la integración de especies invasoras en las comunidades naturales tiende a verse facilitada (Waser y otros 1996). A pesar de que los polinizadores generalistas proporcionan una vía de integración de



plantas invasoras en las redes mutualistas, los efectos sobre la diversidad de polinizadores, por ejemplo, en la pérdida de especialistas, no han sido explorados y son difíciles de prever, debido a la complejidad estructural de las redes. La presente tesis presenta uno de los primeros intentos de caracterizar redes completas de comunidades invadidas en ecosistemas mediterráneos.

Objetivos y estructura de la tesis

El objetivo general de la tesis es el de usar un enfoque de redes mutualistas para estudiar el impacto de las invasiones biológicas en las interacciones entre plantas y polinizadores. La hipótesis general es que las plantas invasoras están bien integradas en las redes planta-polinizador nativas y que alteran las tasas de visitas y patrones de polinización a las plantas nativas. En particular nos preguntamos:

- I. ¿Qué papel juegan los polinizadores nativos en el sistema reproductivo de las plantas invasoras?
- II. ¿Hay competencia por los polinizadores entre las plantas invasoras y las plantas nativas a nivel de comunidad?
- III. ¿Como repercute esto en la estructura de la red planta-polinizador?
- IV. ¿El cambio en la tasa de visitas de los polinizadores a las plantas nativas se refleja en la cadena de transporte de polen?
- V. ¿Qué importancia tiene el contexto, por ejemplo, el tipo de paisaje y los usos del suelo, en el efecto que producen las plantas invasoras sobre las redes planta-polinizador?

Además de este capítulo introductorio la tesis consta de 5 capítulos más. Cuatro hacen referencia a resultados experimentales y el último constituye una discusión general. Para los tres primeros capítulos experimentales utilizamos dos plantas exóticas que han invadido exitosamente muchos ecosistemas mediterráneos, *Carpobrotus affinis acinaciformis* y *Opuntia stricta*. El trabajo de campo se ha llevado a cabo en el Parque Natural del Cap de Creus (Girona). Las dos plantas tienen



flores grandes con abundante polen. *Carpobrotus* (Foto 1.1) es nativo de Sud África y ha sido introducido recientemente (S XIX) a través de jardinería. *Opuntia* (Foto 1.1) es nativa de Centro América y lleva tiempo introducida (S XVII). El cuarto capítulo experimental se ha llevado a cabo en comunidades de rivera rodeadas de una matriz agrícola cerca de Göttingen (Alemania). Para este último capítulo hemos utilizando como modelo de estudio la planta invasora *Impatiens glandulifera* (Foto 1.1). Esta especie fue introducida a principios del S XIX y es nativa del región de los Himalaya.

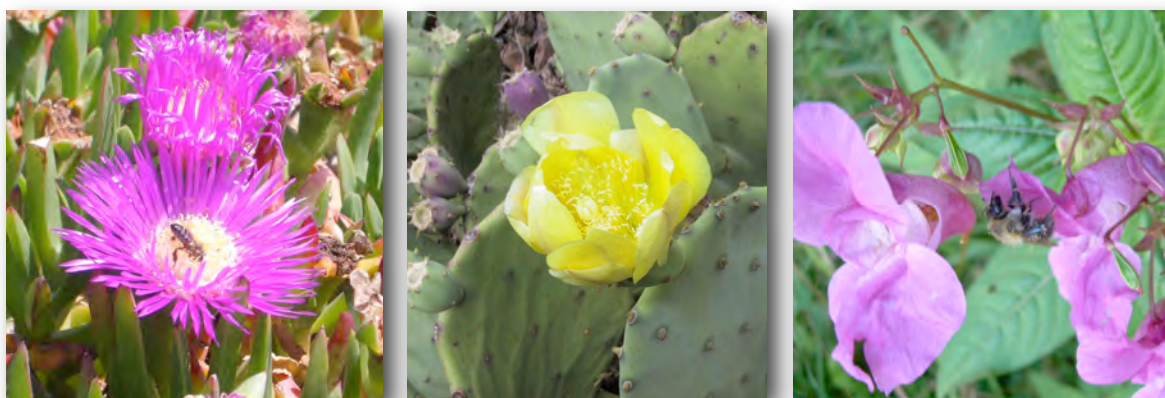


Foto 1.1: Flores de las tres plantas invasoras estudiadas. De izquierda a derecha: *Carpobrotus affine acinaciformis*, *Opuntia stricta* y *Impatiens glandulifera*.

Los 4 capítulos experimentales se presentan en formato de artículo científico pero la bibliografía se ha juntado toda al final de la tesis para evitar repeticiones. Para aumentar la fluidez del texto, a lo largo de la tesis, el nombre de las 3 especies de estudio se limita al del género. Este formato conlleva que la presentación de las especies y área de estudio se repitan en varios capítulos.



Breeding system and pollen limitation of two supergeneralist alien plants invading Mediterranean shrublands¹

Abstract

Many well known invasive plants have entomophilous flowers visited by a wide array of pollinators and are well integrated into the plant-pollinator network. However, their breeding system and the role that pollination services play in the reproductive success of invaders has received little attention. We studied the breeding system and pollen limitation of two entomophilous invasive plants, *Carpobrotus affine acinaciformis* and *Opuntia stricta* in different Mediterranean coastal localities. Both species are to a minor extent self-compatible. Self-compatibility treatments reduced the seed set by at least 75% compared to open pollinated flowers. Both species presented the maximum seed set when pollen from other plants was added. *Carpobrotus* is also wind pollinated. While *Opuntia* was not pollen limited, *Carpobrotus* presented slightly less seeds in open pollination than in hand-cross-pollinated flowers, probably due to its hybrid status which results in a high production of non-viable pollen.

Introduction

Many introduced alien plants establish and spread successfully, thus becoming invaders (Richardson and Pysek 2000). Classic authors (Baker 1955, 1967, 1974; Stebbins 1957) have pointed out that an ideal weed, and therefore an ideal invasive plant, has asexual reproduction, is self-compatible and is a pollinator generalist. Recent studies on invasive plant traits have found that the most widespread and locally abundant invaders have asexual reproduction, air pollen dispersal and/or auto-fecundation (Daehler and Carino 2000; Lloret et al. 2005). The capacity for uniparental reproduction is advantageous not only at the initial establishment stage of invasion by a few founder individuals, but also for the establishment of further populations along the leading edge of an invasion front (Pannell and Barrett 1998). However, many well known invasive plants are entomophilous, pollinated by a wide array of pollinators (Campbell 1989) and receive a high number of visits (Brown and Mitchell 2001; Chittka and Schürkens 2001; Bartomeus et al. 2008). In fact, many entomophilous invasive plants are well integrated in the plant-pollinator

¹ This chapter has been submitted to Biological Invasions. Bartomeus and Vilà (Submitted)



network of the introduced community (Memmot and Waser 2002; Lopezaraiza-Mikel et al. 2007) and except for species with highly specialized pollination systems, such as *Ficus* spp. and orchids, pollinator limitation does not appear to be a major barrier for the spread of introduced plants (Richardson et al. 2000).

When an entomophilous invasive plant gets into a new environment, different scenarios for the interaction with pollinators are possible (Bjerknes et al. 2007). Many invasive plants have open, generalist, actinomorphic flowers and interact with many native pollinator species (Moragues and Traveset 2005; Stout et al. 2006; Bartomeus et al. 2008). On the contrary, some invasive plants have zygomorphic flowers that can attract abundant generalist pollinators such as bumblebees (Chittka and Schürkens 2001; Lopezaraiza-Mikel et al. 2007). Moreover, invasive plant species can also interact with invasive pollinators (Hanley and Goulson 2003) creating supergeneralist complexes (Simpson et al. 2005; Morales and Aizen 2006; but see Olesen et al. 2002). In general, invasive plants are visited by different pollinators in its native range, than in the new invaded areas (Forster 1994; Stout et al. 2006). Therefore, new pollinator interactions could have different efficiency compared to pollinators of the native region. Even if entomophilous invasive plants attract a wide array of pollinators, it is important to ascertain how efficient these pollinators are (Larson et al. 2006; Jakobsson et al. 2007) and if pollination services are needed at all.

Some alien plants, even if visited by pollinators, have a self-compatible breeding system, for example garlic mustard (*Alliaria petiolata*) in North America (Cavers et al. 1979), *Miconia calvescens* in Tahiti (Meyer 1998) and many other invasive species in South Africa (Rambuda and Johnson 2004). Nevertheless, there is little knowledge about whether pollen limitation occurs in invasive species. There are more studies on how seed production in invasive plants depends on insects (Levin and Anderson 1970; Reichard 1994; Harrod and Taylor 1995; Gerlach and Rice 2003; Godfree et al. 2004) than studies testing pollen limitation in invasive plants. In native plants pollen limitation is spatially variable (Ahsman et al. 2004; Knight et al. 2005) and this seem to also be the case for invasive species. *Cistus scoparius*, a European plant invading western Northamerica, is pollen limited in locations with lower pollinator visitation, but not in high visitation sites (Parker 1997; Parker and Haubenask 2002). Similarly, the skunk vine (*Paedria*



foetida) invading native habitats in Florida is pollen limited in certain populations, depending on pollinator composition (Liu 2006). These studies suggest that the effect of pollinators on seed production in invaders is highly context specific.

Moreover, interpopulation variation in the breeding system is common (Dieringer 1999; Lee et al. 2000), especially, in alien species populations that arise from a few founder individuals (Sakai et al. 2001). We studied the breeding system and pollen limitation of the two entomophilous invasive taxa, *Carpobrotus affine acinaciformis* (*Carpobrotus* hereafter) and *Opuntia stricta* (*Opuntia* hereafter) in different localities. Vegetative reproduction is important in both species for the persistence of their populations, but seedling recruitment is essential to expand their area of distribution and to spread into new areas (Gimeno and Vilà 2002 for *Opuntia*; Vilà and D'Antonio 1998 for *Carpobrotus*). Both species are entomophilous supergeneralist species (Bartomeus et al. 2008) and studies conducted in other regions have found that they are slightly self-compatible (Suehs et al. 2004b for *Carpobrotus*; Spears 1987 for *Opuntia*). The case of *Carpobrotus* is particularly interesting because different levels of introgression between *C. edulis* and *C. acinaciformis* in different localities could affect its breeding system (Suehs et al. 2004a). Our hypothesis is that these two case study invaders are not pollen limited and even if they are self-compatible, pollinators contribute largely to the seed set.

Methods

Study area

Our study area was located in coastal Mediterranean shrublands in the Natural Park of Cap de Creus (Catalonia - NE Spain). This area is characterised by cool wet winters and warm dry summers. Mean temperatures of the coldest (January) and hottest (August) months in 2005 were 6° C and 23° C, respectively, and the annual precipitation was 450 mm (www.meteocat.com). Our study involved the most abundant invasive plants in the Park. *Carpobrotus* is present in coastal communities and *Opuntia* in hilly stony slopes.



For each species we selected 3 representative invaded areas of 50 x 50m. These areas were at least 3 Km apart. Cover of the invaders was from 15 to 35%, typifying an initial invasion stage. *Carpobrotus* and *Opuntia* coexisted with 17 and 16 co-flowering species, respectively (Appendix I).

Previous plant-pollination analyses showed that the native plant community was visited by Coleoptera and Hymenoptera and also by certain Diptera and Lepidoptera (Appendix I). In contrast *Carpobrotus* and *Opuntia* were mainly visited by Coleoptera and Hymenoptera. In total, 23 and 17 different taxa visited *Carpobrotus* and *Opuntia*, respectively (Appendix II). Almost no pollinators were exclusive to *Carpobrotus* which was visited by 43.4 % of the observed insect taxa. *Opuntia* was visited by 30.9 % of the observed insect taxa (Bartomeus et al. 2008).

Invasive species

Carpobrotus (Aizoaceae) are crawling, mat forming, succulent herbs with fast clonal growth (Vilà and D'Antonio 1998). Native to South Africa, they are invasive in almost all Mediterranean regions of the world. They were introduced in Spain for gardening and soil fixation at the beginning of the twentieth century. The main impacts are smothering, reduced regeneration of native flora, and changes to soil pH and nutrient regimes, competing aggressively with native species (D'Antonio and Mahall 1991; D'Antonio 1993). In the study area *Carpobrotus* may be hybrids between *C. edulis* and *C. acinaciformis*. We will therefore follow Suehs et al's (2004a) nomenclature and refer to them as the hybrid complex *Carpobrotus affine acinaciformis*. These putative hybrids have flowers that are solitary and 8-10 cm in diameter, range in colour from yellow to purple and have a generalist pollination system (Vilà and D'Antonio 1998). Flowering lasts from March to June (Sanz-Elorza et al. 2006). Fruit are fleshy, indehiscent and edible, 35 mm in diameter. Each fruit produces hundreds of seeds embedded in a sticky, sweet, jelly-like mucilage.

The breeding system of *Carpobrotus* sp hybrids and putative parents has partially been analysed in other Mediterranean communities. *C. edulis* is slightly agamospermic and self-fertile (Suehs et al.



2004b; Vilà et al. 1998). In California, *C. edulis* hybridises with *C. chilensis* (Vilà and D'Antonio 1998) and hybrids are self-compatible. In France, *C. edulis* hybridises with *C. acinaciformis* resulting in the complex *C. affine acinaciformis* forming stands with different levels of introgression. *Carpobrotus aff. acinaciformis* is slightly self-compatible (Suehs et al. 2004b). In the study area, our target taxa had a phenotype like *C. affine acinaciformis* taxa in southern France (Suehs et al. 2004a).

Opuntia stricta (Cactaceae) is a succulent perennial spiny plant introduced to Spain from Central America in the sixteenth century. *Opuntia* can dominate the vegetation of rocky outcrops, displacing native species. It is commonly cultivated as an ornamental and invades sunny sandstone hillsides and abandoned orchards (Vilà et al. 2003). Plants can reach 1.5 m high and have yellow flowers with abundant pollen that are 5-10 cm in diameter. Flowering takes place from June to July (Sanz-Elorza et al. 2006). Fruits are obovoid and contain dozens of 4-6 cm long, 2.5-4 cm diameter seeds (Gimeno and Vilà 2002). Seeds are numerous and 4-5 mm long. The genus *Opuntia* has very heterogeneous breeding systems. Autogamy is rare, most species are self-compatible and some need outcrossing to set seed (Reyes-aguero 2006). It has been documented that levels of self-pollination could change between populations (Bianchi et al. 2000). In Florida, *O. stricta* presents a high level of self-pollination and no pollen limitation (Spears 1987).

Breeding system

In each site and for each invasive species we assigned the following pollination treatments following Kearns and Inouye (1993) and Neal (2004) protocols: (1) Spontaneous self-pollination: bagged flowers (0.2 mm size pore bags except for anemogamy treatment, see below) to avoid any pollen transfer; (2) Facilitated self-pollination: hand-pollination with pollen from its own flower. Flowers remained bagged before and after the hand-pollination treatment. (3) Anemogamy: bagged flowers with 2.5 mm size pore, this diameter permits the passing of pollen but excludes pollinators; (4) Xenogamy: bagged flowers with pollen added from other populations to ensure that pollen belongs to different individuals. These flowers were also bagged to ensure no pollen



transfer; (5) Control: open pollinated flowers which were not manipulated. For treatments involving hand pollination, this was performed using cotton swabs on fully receptive stigmas. To assure pollen viability, all manual pollen transfer was done collecting fresh pollen 15 minutes before hand-pollination.

For *Carpobrotus*, as it was not possible to distinguish between different genetic individuals, 40 flowers were randomly assigned to each treatment in each site. For *Opuntia*, 20 individuals per site were used and two flowers per individual were randomly assigned to each treatment.

At the end of the experiments, some bags had disappeared as fruits were consumed by frugivores but we collected data from at least 20 bags per treatment and site. After fruit maturation, fruits were collected and opened in the laboratory for seed counting. In total we counted seeds from 498 *Carpobrotus* fruits and 435 *Opuntia* fruits. Differences in the seed set between treatments were tested with an ANOVA using the 5 treatments as fixed factors, and site as the random factor. For *Opuntia*, plant was also included as a random factor. Post-hoc Tukey tests were conducted to assess differences between treatments.

Results

All fruits collected had seeds. The number of seeds differed between treatments both in *Carpobrotus* and in *Opuntia* (Figure 2.1). Post-hoc Tukey tests showed that both species presented the maximum seed set when pollen from other plants was added (xenogamy), but in *Opuntia* this treatment was not significantly different from open pollinated flowers. Both species are self-compatible, but this treatment reduced the seed set by at least 75% compared to open pollinated flowers. While *Carpobrotus* presented a similar amount of seeds due to anemogamy as in open pollinated flowers, in *Opuntia*, anemogamy reduced the seed set to half. Facilitated self-pollination almost doubled the seed set compared to spontaneous self-pollination.



We did not find any site or individual plant effect, indicating that overall spatial differences were not significant. However, there were significant interactions with the pollination treatment, indicating slight differences in the breeding system between sites and even among plants (Table 2.1).

Table 2.1: ANOVA of *Carpobrotus aff. acinaciformis* and *Opuntia stricta* seed production between different pollination treatments, taking into account the site for *C. aff. acinaciformis* and site and plant for *O. stricta* as random factors.

<i>Carpobrotus</i>	d.f.	F	P
Treatment	4	18,67	0,001
Site	2	0,11	0,8
Treatment x site	8	4,6	0,001
Error	482		

<i>Opuntia</i>	d.f.	F	P
Treatment	4	86,73	0,001
Site	2	0,92	0,5
Plant	89	0,78	0,7
Treatment x site	55	1,3	0,001
Treatment x plant	2573	1,4	0,001
Error	434		

Discussion

Although the two case study invasive plants have the biggest flowers in the community, they were mainly visited by the same pollinators as natives (Bartomeus et al. 2008). In both species local pollinators contributed largely to the seed set. Cross-pollinated *Carpobrotus* flowers had the highest seed set in all three sites indicating that there are no significant differences between populations. On average in our study sites, cross-pollination levels were 50% lower than in south France (Suchs et al 2004b). Differences between studies could be associated to different levels of introgression. Alternatively, differences could be due to the fact that French populations were cross-pollinated with individuals of the same population, while we used plants from other populations therefore increasing the probability of outcrossing.



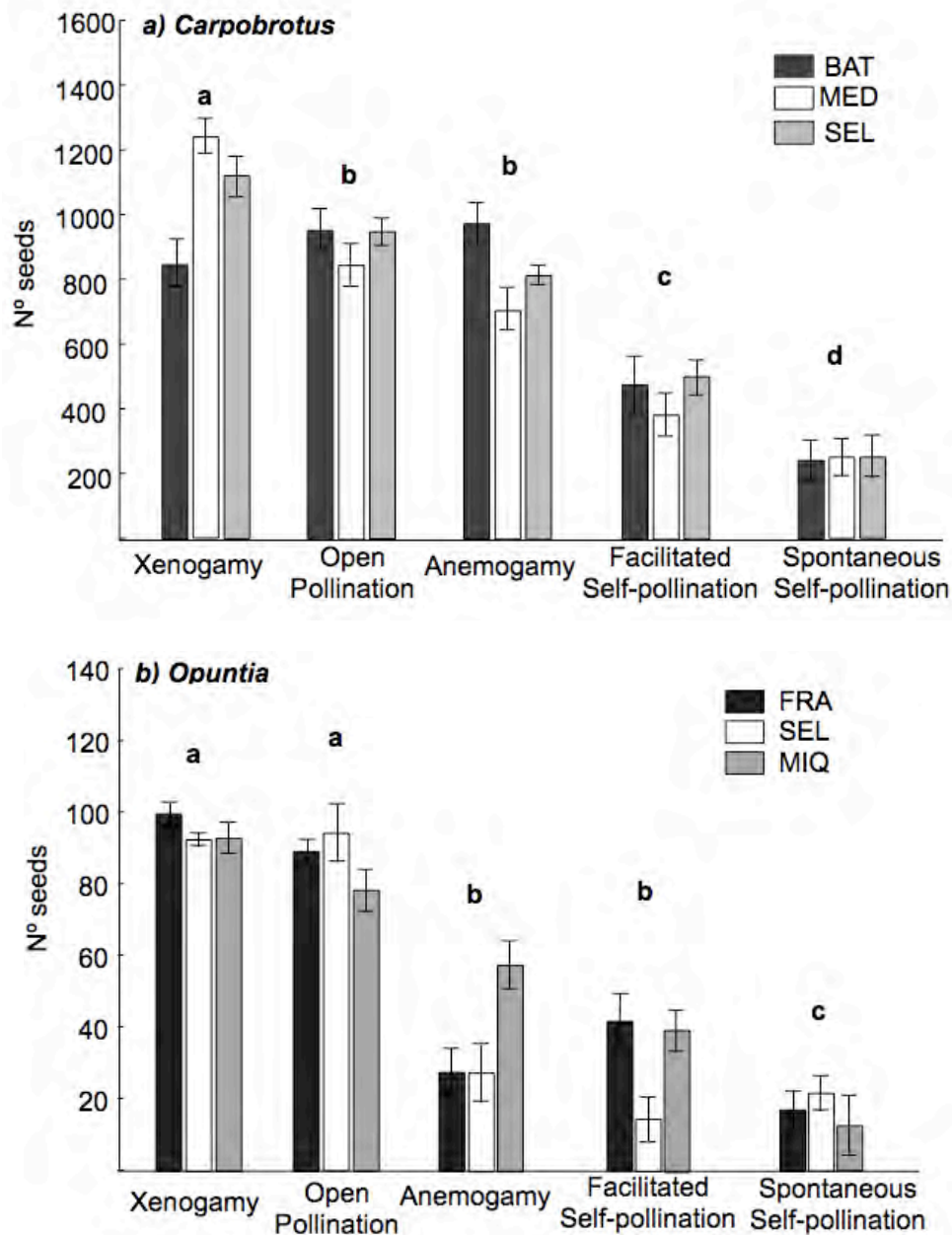


Figure 2.1: Mean \pm SE of the number of seeds per fruit produced by *Carpobrotus aff. acinaciformis* (a) and *Opuntia stricta* (b) in 3 sites (BAT, MED and SEL) for each pollination treatment: xenogamy (hand-pollination with pollen from other localities); open pollination; anemogamy (wind pollination); facilitated self-pollination (hand-pollinated stigmas with the pollen of the same flower); and spontaneous self-pollination (bagged flowers). Different numbers on columns indicate significant differences between breeding-systems.

Recent studies have reported that pollinators visiting *Carpobrotus* carry high amounts of *Carpobrotus* pollen (Jakobson et al. 2008; Bartomeus et al. submitted). We thus expected that plants should not be pollen limited. Nevertheless, open pollinated *Carpobrotus* flowers presented a lower seed set than cross-pollinated flowers. Our results indicate that *Carpobrotus* was slightly



pollen limited. We have to take into account that the main pollinators visiting *Carpobrotus* are beetles that have a low flower visitation rate, and even if present in high abundance, they spend long periods of time on single flowers (Bosch 1992). This, overall, reduces effective cross-pollination. Moreover, the production of aborted pollen can be high in hybrids (Suehs et al. 2006). In the populations studied, on average aborted pollen was 30% (Bartomeus, personal observation) and this could decrease the quality of the pollen deposited on stigmas.

On the contrary, *Opuntia* presented similar levels of seed production in cross-pollination treatment as in open pollinated flowers, indicating that pollinators are very efficient and the species is not pollen limited. In its native range, *Opuntia* sp is strongly associated with bee pollination and co-evolution with at least two genera is suggested (Grant et al. 1979; Reyes-aguero 2006). Moreover, in the native range insects less than 1.5 cm are not able to pollinate *Opuntia* sp flowers (Grant and Hurd 1979). In our study site, *Opuntia* is visited by many bee species (Bartomeus et al. 2008), including long visits from honeybees, that are kept by man for honey production, and carpenter bees. Both are big pollinators capable of providing a proper pollination. In the Mediterranean, *Opuntia* flowers late in the season when flowering of most of the other bee pollinated species (e.g. *Rosmarinus officinalis*, *Lavandula stoechas*) is over, hence reducing plant species competition for pollinators.

Both *Carpobrotus* and *Opuntia* are self-compatible. Facilitated self-pollination produced more seeds than spontaneous self-pollination, but both treatments produced less than half the number of seeds compared to open pollinated flowers. It should be kept in mind that the mechanistic movement of an insect within a flower contributes to self-pollinate it. This was reported in California, where 80% of seeds in the congener *C. chilensis* arose from trips involving self-pollination (Vilà et al.1998)

Based on previous studies, we expected high self-pollination in *Opuntia* (Spears 1989). On the contrary, our three populations demonstrated low levels of self-pollination that increased when



facilitated mechanically. Other *Opuntia* species present different levels of self-fertility between populations (Bianchi et al. 2000).

To our knowledge this is the first study testing anemogamia in *Carpobrotus* and *Opuntia*. Anemogamy was not significant in *Opuntia*, but in *Carpobrotus* wind pollinated plants had as much seed production as open pollinated flowers. *Carpobrotus* flowers do not fit into a typical wind pollination scenario (Proctor et al. 1996). Nevertheless, these flowers have an open bowl shape with exposed stigmas and a high production of small pollen (Blacke 1969). Invaded sites are very windy and as already stated in other entomophilous species, wind can contribute to pollen dispersal (Dafni and Dukas 1986; Bullock 1994).

Conclusions

Invasion processes are subjected to bottleneck events that could constrain the variability in the breeding system (Sakai et al. 2001). In the introduced community invasive plant species face a new reproductive situation that is important in order to understand the mechanisms involved in the invasion process. The success of *Carpobrotus* and *Opuntia* as invaders in Mediterranean coastal shrublands could be explained by their clonal reproduction, a generalist pollination syndrome that allows a wide range of pollinators to visit their flowers and by a certain degree of self-pollination. In *Opuntia*, these conditions confer no seed limitation. In *Carpobrotus* wind can also contribute to pollen dispersal as much as pollinators, nevertheless this taxa is pollen limited probably due to its hybrid status resulting in a large amount of non-viable pollen.



Contrasting effects of invasive plants in plant-pollinator networks²

Abstract

The structural organization of mutualism networks, typified by interspecific positive interactions, is important to maintain community diversity. However, there is little information available about the effect of introduced species on the structure of such networks. We compared non-invaded and invaded ecological communities, to examine how two species of invasive plants with large and showy flowers (*Carpobrotus affine acinaciformis* and *Opuntia stricta*) affect the structure of Mediterranean plant-pollinator networks. To attribute differences in pollination to the direct presence of the invasive species, areas were surveyed that contained similar native plant species cover, diversity and floral composition, with or without the invaders. Both invasive plant species received significantly more pollinator visits than any native species and invaders interacted strongly with pollinators. Overall, the pollinator community richness was similar in invaded and non-invaded plots, and only a few generalist pollinators visited invasive species exclusively. Invasive plants acted as pollination super-generalists. The two species studied were visited by 43% and 31% of the total insect taxa in the community, respectively, suggesting they play a central role in the plant-pollinator networks. *Carpobrotus* and *Opuntia* had contrasting effects on pollinator visitation rates to native plants: *Carpobrotus* facilitated the visit of pollinators to native species, whereas *Opuntia* competed for pollinators with native species, increasing the nestedness of the plant-pollinator network. These results indicate that the introduction of a new species to a community can have important consequences for the structure of the plant-pollinator network.

Introduction

The establishment and spread of introduced alien species modifies the structure and stability of ecological communities (Richardson and Pyšek 2000). The most well-known effects of invasive plant species on communities involve direct competition for nutrients, light, space or water, and the consequent loss of plant species diversity (Lodge 1993; Levine et al. 2003). In contrast, there is little information available about their effects on other trophic levels such as the bottom-up

² This chapter is published in *Oecologia*; Bartomeus, Vilà and Santamaría (2008)



relationships. However, there is some evidence to suggest that invasive plants could cause disruptions to mutualistic interactions such as between plant and pollinators (Traveset and Richardson 2006).

A few studies on pollinator competition prompted by invasive plants have examined specific plant-pollinator interactions, in which visitation rates or diversity of pollinators were compared for particular native species present in invaded and non-invaded areas. Results of specific plant-pollinator interaction studies have yielded inconsistent results and so we can not generalize that alien plants always affect the pollination success of native plants (Bjerknes et al. 2007). For example, the invader *Lythrum salicaria* reduces pollinator visitation and the seed set of its native congener *L. alatum* under experimental conditions (Brown et al. 2002). Similarly, *Impatiens glandulifera* halves the seed set of the native *Stachys palustris* through pollinator competition (Chittka and Schürkens 2001). Although *Carpobrotus spp.* has no effect on the pollination of the rare plant *Dithyrea maritime* in southern California (Aigner 2004), in the Balearic Islands it has a competitive effect (i.e. fewer visits) on *Lotus cytisoides*, but a facilitative effect (i.e. more visits) on *Cistus salvifolius* and *Anthyllis cytisoides*, and no effect on *C. monspeliensis* (Moragues and Traveset 2005).

Although the above examples demonstrate some pollination changes regarding a focal plant species in the presence of a plant invader, it is important to understand how these changes affect the entire community's plant-pollinator interaction network. Mutualistic networks are extremely important for the diversity and stability of the community because they are involved in coevolution processes where multiple species rely on each other to succeed (Jordano 1987, Bascompte and Jordano 2007). Some animal-pollinated invasive plants may become integrated into plant-pollinator networks of the recipient community (Memmott and Wasser 2002) and may even be visited by endemic super generalist pollinators (Olesen et al. 2002). It is, therefore essential to understand how invasive plants affect not only single plant-pollinator interactions but the whole plant-pollinator network. Only one study has examined how visitation rates differ between invaded and non-invaded sites. Lopezaraiza-Mikel et al. (2007) compared sites invaded by *Impatiens glandulifera* with sites where *I. glandulifera* was experimentally removed. They found that the



invader acted as a magnet species, invaded sites being more visited than those in which the invader was removed from the community.

More recent work has explored the inherent structure of plant-pollinator networks. Plant-pollinator networks are not randomly organized; they have a nested structure (i.e. nestedness) meaning that there is a core of generalist plant and pollinator species, and an asymmetric dependence between them. Some specialized pollinators visit generalist plant species and vice-versa, specific plant species are visited by generalist pollinators (Bascompte et al. 2003; 2006). The characteristics of plant-pollinator networks differ along environmental gradients such as humidity, insularity or latitude (Olesen and Jordano 2002; Ollerton and Cranmer 2002; Devoto et al. 2005). However, the effect of biotic controls on plant-pollinator networks, such as the consequences of adding a new plant species (e.g. due to biological invasions) to the community have been far less explored. Moreover, no study has empirically investigated how network nestedness changes after a new species is introduced to a community.

We present here results of a field replicated comparative study on the effects of plant invasions on plant-pollinator interaction networks. Our hypothesis is that an introduced generalist invasive plant will establish many interactions with resident pollinators and consequently some interactions with native plant species will be lost. In principle this would increase network nestedness as a greater number of pollinator species interact with fewer plant species in the community. To test this hypothesis, we studied *Carpobrotus affine acinaciformis* and *Opuntia stricta*, which have invaded Mediterranean coastal communities. Both species have showy, large flowers but contrasting phenologies that could result in different effects on plant-pollinator networks. *Carpobrotus* flowers in spring, when most native species flower. In contrast, *Opuntia* flowers in early summer, when a few late-flowering natives bloom. Mediterranean communities tend to have more flowers than insects in early spring, whereas in late spring there are more insects than flowers. Thus, in early spring one would expect plants to compete for pollinators, whereas in summer pollinators would compete for flowers (Shmida and Dafni 1989; Petanidou and Lamborn 2005). We expected *Carpobrotus* to have a higher impact on the pollination network than *Opuntia*. Specifically, we asked, 1) Which pollinators visit invasive plants, as compared to native



plants? 2) Do pollinator diversity, composition and visitation rates differ between invaded and non-invaded communities? and 3) Does the structure of the plant-pollinator network (i.e. composition, strength of plant species, and nestedness of plant-pollinator interactions) change between invaded and non-invaded communities?

Methods

Study area and study species

Our study area was in coastal Mediterranean shrublands in the Natural Park of Cap de Creus (Catalonia - northeastern Spain). This area is characterized by cool wet winters and warm dry summers. Mean temperatures of the coldest (January) and hottest (August) months in 2005 were 6° C and 23° C, respectively, and the annual precipitation was 450 mm (www.meteocat.com). Our study involved a pollinator survey of the native flora and the two most abundant invasive plants in the park.

Carpobrotus (Aizoaceae) are crawling succulent herbs with fast clonal growth (Vilà and D'Antonio 1998). They are native to South Africa and are present in almost all Mediterranean regions of the world. They were introduced in Spain for gardening and soil fixation at the beginning of the twentieth century. In the study area *Carpobrotus* may be hybrids between *C. edulis* and *C. acinaciformis*. We will therefore follow Suehs et al's. (2004a) nomenclature and refer to them as the hybrid complex *Carpobrotus affine acinaciformis*. These putative hybrids have flowers that are solitary and 8-10 cm in diameter, range in color from yellow to purple and have a generalist pollination system (Vilà and D'Antonio 1998). Flowering lasts from March to June (Sanz-Elorza et al. 2006).

Opuntia stricta (Cactaceae) is a succulent perennial spiny plant introduced to Spain from Central America in the sixteenth century. It grows in sunny sandstone hillsides and is commonly cultivated as an ornamental. It can reach 1.5 m high and has big yellow flowers (5-10 cm in diameter). Flowering takes place from June to July (Sanz-Elorza et al. 2006).



Field sampling

In spring 2005, we delineated three sites per study species and established two paired 50 x 50 m plots in each site, one invaded and the other non-invaded separated on average by 300 m. Paired sites were at least 3 km apart from each other. To exclude possible indirect effects of the invasive plants on pollinator presence due to changes in plant species diversity (Kunin 1997) we chose areas that exhibited initial stages of invasion and selected pairs of plots with similar plant species diversity, dominant species and vegetation cover. Plant species diversity was calculated using Shannon index $H' = -\sum |P_i \log_{10} P_i|$; where P_i is the frequency of occupation of species i . Plant community cover was determined by the point-intercept-method in four parallel 50 m transects (Table 3.1). Variation in plant species richness was not significant (invaded vs. non-invaded: 10.3 ± 0.7 vs 12 ± 2.3 , $t = -2.5$, $p = 0.13$ for *Carpobrotus* sites and 10.3 ± 1.3 vs 10.3 ± 0.8 , $t = 0.1$, $p = 0.9$ for *Opuntia* sites). Field work was conducted on sunny days with little wind and with temperatures higher than 15° C. Sites were sampled every 2 weeks during the entire flowering period of both species: six times from April to May for *Carpobrotus* sites and four times from June to early July for *Opuntia* sites.

Within each site, insect pollinator counts were made along two parallel 50 m permanent transects. To avoid oversampling of the most abundant plant species we limited our observations to a total of six observation areas per flowering species. In each observation area we focused only on one flowering species. The observation areas were approximately 30 x 30 cm and were located along transects at a 2 m interval. To reduce any temporal bias in observations along transects, we started each day's sampling at a different random initial point along each transects. In each observation areas, we counted the number of flowers, and recorded and identified all insects that visited the flowers within one minute of observation. We captured unknown visitors during a maximum of three additional minutes for later identification. Voucher specimens were deposited at CREAM (Center for Ecological Research and Forestry Applications). We only recorded an insect as a visitor if it touched the reproductive organs of the flower.





Table 3.1: Characteristics of *Carpobrotus affine acinaciformis* and *Opuntia stricta* in invaded and non-invaded plots. See text for details on methods.

Site	Plot	Sampling period	Target invader	Target invader cover (%)	Total cover (%)	Dominant native species	Dominant species cover (%)	Shanon index	No. flowering species	Sorensen index
BAT	Non-Invaded	April-May			180	<i>Lavandula stoechas</i> , <i>Thimelea hirsuta</i>	70,5	1,06	9	0,95
	Invaded	April-May	<i>Carpobrotus</i>	46,5	167	<i>T. hirsuta</i> , <i>L. stoechas</i>	39,5	1,13	10	
MED	Non-Invaded	April-May			162	<i>Rosmarinus officinalis</i> , <i>Cistus monspeliensis</i>	35,5	1,07	11	0,72
	Invaded	April-May	<i>Carpobrotus</i>	39,0	140	<i>C. salvifolius</i> , <i>R. officinalis</i>	23,0	1,13	14	
FAR	Non-Invaded	April-May			149	<i>C. monspeliensis</i> , <i>R. officinalis</i>	36,5	1,18	11	0,87
	Invaded	April-May	<i>Carpobrotus</i>	25,5	139	<i>C. salvifolius</i> , <i>R. officinalis</i>	29,0	1,24	12	
SEL	Non-Invaded	June-July			147	<i>L. stoechas</i> , <i>Sedum sedifforme</i>	52,5	0,95	12	0,75
	Invaded	June-July	<i>Opuntia</i>	12,5	134	<i>L. stoechas</i> , <i>Psoralea bituminosa</i>	24,5	0,94	11	
FRA	Non-Invaded	June-July			120	<i>D. carota</i> , <i>Aetheorrina bulbosa</i>	35,0	1,05	10	0,64
	Invaded	June-July	<i>Opuntia</i>	24,5	132	<i>D. carota</i> , <i>A. bulbosa</i>	23,5	1,04	11	
MIQ	Non-Invaded	June-July			130	<i>A. bulbosa</i> , <i>P. bituminosa</i>	40,0	0,91	9	0,80
	Invaded	June-July	<i>Opuntia</i>	15,0	118	<i>D. carota</i> , <i>A. bulbosa</i>	27,5	1,08	9	

Our sampling protocol allowed us to standardize the time spent observing each plant species compared to classical transect walks. Each species was sampled for a total of 36 minutes per site. In total, each site was surveyed for pollinators for more than 6 h.

To ascertain whether differences in pollinator activity were due to differences in flower abundance, we estimate the number of flowers of each plant species in the vicinity of each transect. Each sampling day when pollinator sampling was finished, we determined the total number of flowers or inflorescences (flower units hereafter) per plant species in 1 m² areas located at 1-m intervals along the transects. The total number of flower units in paired invaded and non-invaded plots did not differ for either species (Wilcoxon test paired comparisons for *Carpobrotus* $p = 0.3-0.5$ and for *Opuntia* $p = 0.2-0.5$). We therefore assumed pollinator activity in invaded and non-invaded plots was independent of differences in native floral abundance.

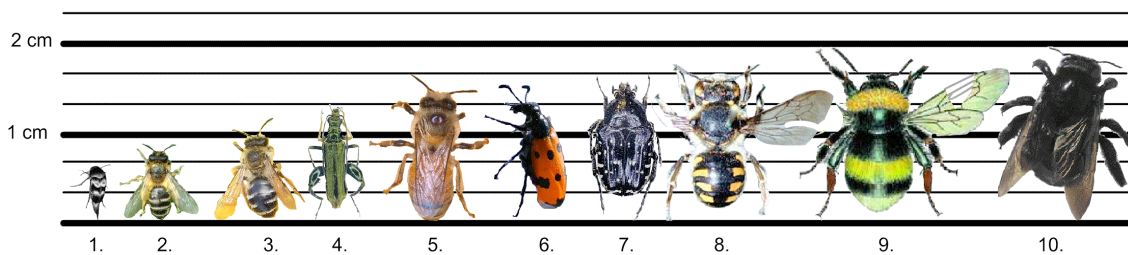


Figure 3.1: Common genera of pollinators in our *Carpobrotus* and *Opuntia* networks. 1. *Mordellistena*, 2. *Andrena*, 3. *Halictus*, 4. *Oedemera*, 5. *Apis*, 6. *Myrabilis*, 7. *Oxythyrea*, 8. *Anthidium*, 9. *Bombus*, 10. *Xylocopa*.

Plant-pollination network analysis

With the data collected we constructed a plant-pollinator interaction network for each of the 12 plots. Networks were represented as two-dimensional matrices, in which rows represented the plants and columns the pollinator species. We constructed two matrices per plot, one in which cell values represented the presence or absence of the interaction and another in which cells were the frequency of visits observed for each plant-pollinator interaction.



To test whether the two invasive species received a number of visits comparable to average visitation on a particular native species within each invaded plot, we compared the visitation frequency received by the invader with a normal distribution fitted to the number of visits to native species, with a Z -statistic.

To explore whether invaded and non-invaded plots differed in pollinator assemblage, we calculated the Sorensen similarity index $S = (2 \times C) / (2C + A + B)$; where C is the number of pollinator species that were common in both plots, A is the number of pollinators only present in invaded plots, and B is the number of pollinators present only in non-invaded plots. The Sorensen index ranges from 1 (identical assemblages) to 0 (maximally dissimilar assemblages).

Differences between invaded and non-invaded plots in pollinator species richness, frequency of visits of the main pollinator orders, and frequency of visits to native plants were compared with paired t tests. To account for the limited power associated to the low sample size ($n = 3$ networks per treatment), we also report marginally significant differences ($\alpha = 0.1$). The limited sample size is largely justified by the considerable effort involved in the simultaneous characterization of several whole plant-pollinator networks in an area where a reference pollinator collection does not exist. Statistical analyses were carried out using STATISTICA (StatSoft 2001).

We tested whether invasion influenced pollinator visitation rates (i.e., number of visits per flower) to native species with a generalized linear mixed model (GLMM; PROC MIXED in SAS (SAS Institute 2001). The model was visitation rate = presence of the invader (yes or no) + plot + plant family + plant species nested within family + error. We considered family and plot random factors, and species and presence or absence of the invader fixed factors. In this way, we could test whether the invaders affected the visitation rates of native plant species while accounting for the clustering of plants according to taxonomy and plot.

We also compared similarities of the interaction scenarios (i.e. identities and frequencies of plant-pollination interactions) between invaded and non-invaded visitation matrices by constructing one



similarity matrix estimated with Kulczynski's distance coefficient ($K = 1/2(W/A+W/B)$; where W is the sum of the minimum visits of the various species, this minimum being defined as the visits at the site where the species is the rarest and, A and B are the sums of the visits of all species at each site, respectively). The index was calculated between all pairs of plots (Legendre and Legendre 1998; Vázquez and Simberloff 2003). This index was semi-metric, meaning that it accounted for differences in contribution to the coefficient between abundant species and rare species. We used the resulting pair-wise similarity matrix as input for non-metric multidimensional scaling (MDS), an ordination technique used to provide a visual representation of the pattern of proximities among plots (Legendre and Legendre 1998; Vázquez and Simberloff 2003). To test differences in the array of interactions between plots we performed a Wilcoxon test for each pair of plots where all paired plant-insect interactions in the two plots, including empty cells, were used as input.

To explore in depth the similarity of plant-pollinator interactions, we calculated the number of common plant-pollinator interactions (I_c) and the number of plant-pollinator interactions that occurred in only one of the paired plots (I_e). In invaded plots we could distinguish between exclusive pollinator-invasive species interactions (I_i) and exclusive pollinator-native species interactions (I_n). Therefore, in invaded plots: $I_e = I_i + I_n$.

We further analyzed the structure of the matrix with the index of matrix nestedness (N) and the importance of each plant species in terms of interaction strength. Matrix nestedness is an estimation of the network organization (Bascompte et al. 2003) and is calculated from Temperature (T) as $N = (100-T)/100$. Values range from 0 (random structure) to 1 (maximum nestedness). To calculate T , we organized the matrix in rows of pollinators and columns of plants, ordering the entries from those presenting the most to the least interactions. This maximally packed matrix was used to calculate the isocline of perfect nestedness for each matrix. The absence of a pair-wise interaction below the isocline or the presence of a pair-wise interaction above the isocline was then recorded as unexpected. Therefore, T is the measure of physical disorder calculated from the normalized measure of global distance from unexpected records to the isocline. To assess the significance of N for each matrix, we compared the observed value of N with a benchmark provided by Null Model 2 in Bascompte et al. (2003) in which each cell in the interaction matrix has the following probability $(P_{ri} + P_{ci})/2$, where P_{ri} is the fraction of



interactions of row i and P_{ci} is the fraction of interactions of the column i equivalent. We generated 1000 random matrices with this null model. Analyses were conducted with the ANINHADO software (Guimaraes Jr and Guimaraes 2006). T values for plants represented how far from the nested pattern each plant species was, and idiosyncratic T values were used to assess changes in the community. Differences in N between invaded and non-invaded plots for each invader species were compared with a paired t test.

The strength of each plant species in the matrix is a measure of the dependence of pollinators on each particular plant species (Bascompte et al. 2006). Strength is defined as the sum of pollinator dependencies on a particular plant species. Dependence of a pollinator on a plant species is the fraction of all visits by a particular pollinator species to a particular plant ($D_{a,p} = V_{a-p} / V_a$, where $D_{a,p}$ is the dependence of pollinator a on plant species p , V_{a-p} is the number of visits of pollinator a to plant p and V_a is the total number of visits of pollinator a to all plant species in the community). We tested for differences in the strength of plant species between invaded and non-invaded plots with a paired t test. Furthermore, to test whether the two invasive plant species had a higher strength than native species, within each site we compared the invasive species strength with a normal distribution fitted to the strength of the native species by means of a Z statistic.

Results

Pollinators on invader versus native plant species

We observed a total of 23 and 17 insect taxa on *Carpobrotus* and *Opuntia*, respectively. Both species were visited by several species of Coleoptera (e.g., *Oedemera lurida* and *Oxythrea funesta* on *Carpobrotus* and *Myrabilis quadripunctata* on *Opuntia*) and Hymenoptera (e.g., *Bombus terrestris*, *Anthidium sticticum* to *Carpobrotus* and *Xylocopa violacea*, *Apis mellifera* to *Opuntia*, Figure 3.1). The native plants were also visited by some Diptera and Lepidoptera. Almost no pollinators were exclusive to *Carpobrotus*, which were visited by 43.4% of the observed insect taxa. *Opuntia* was visited by 30.9% of the observed insect taxa. *Xylocopa violacea* (i.e., Carpenter



bees) exclusively visited *Opuntia* plants and were a frequent pollinator (19.5% of the total visits to *Opuntia*).

Both invader species received on average more visits than any native species (z tests: $p < 0.001$ in all sites). *Carpobrotus* received $27.8 \pm 7.2\%$ (mean \pm SE) of the total visits observed. On average, native species in invaded plots received $7.61 \pm 1.23\%$ of the visits. *Opuntia* received $44.1 \pm 11.2\%$ of the observed visits, whereas on average, native species received $6.16 \pm 2.33\%$.

Pollinator structure in invaded versus non-invaded communities

In total we found 53 and 55 different pollinator taxa in invaded and non-invaded *Carpobrotus* and *Opuntia* sites, respectively. There were differences in the composition of invaded and non-invaded plots measured with the Sorensen index; $S = 0.52 \pm 0.02$ for *Carpobrotus* sites and $S = 0.54 \pm 0.04$ for *Opuntia* sites. However, the presence of an invasive species did not affect pollinator species richness (invaded vs. non-invaded: 29.33 ± 3.71 vs. 20.33 ± 1.21 , $t = 2$, $p = 0.18$ for *Carpobrotus* sites; 23.67 ± 1.20 vs. 21.00 ± 2.00 , $t = 1.02$, $p = 0.29$ for *Opuntia* sites). We only found significantly more Coleoptera visits ($t = -5.46$; $p = 0.03$) in *Carpobrotus*-invaded plots compared to non-invaded plots. This difference was principally due to the frequency of visits to the invasive species and to Asteracea and Cistacea. There was no detectable difference in the insect orders observed in *Opuntia*-invaded and non-invaded sites, probably because of the large variability among plots (Figure 3.2; paired t tests, $p > 0.10$).

The frequency of visits to the invasive and native species as a whole did differ. There were marginally more visits in invaded than in non-invaded *Carpobrotus* plots (159.33 ± 22.26 vs. 83.33 ± 6.76 , $t = 2.97$, $p = 0.10$). In the invaded plots, visitation rates increased significantly in more than 70% of the native species (GLMM test; Fixed effects, Presence of the invader: $F_{1,29} = 6.23$; $p = 0.02$, Species within families: $F_{14, 1} = 18.01$; $p = 0.18$; Random effects, Plot: $Z = 0.93$; $p = 0.35$; Family $Z = 1.21$; $p = 0.23$) In contrast, the overall number of visits to *Opuntia* plots did not differ



between invaded and non-invaded plots (95.33 ± 10.14 vs. 70.67 ± 4.10 , $t = 2.51$, $p = 0.13$). However, visitation rates decreased in more than 60% of the native species in invaded plots (Fixed effects; Presence of the invader: $F_{1,27} = 4.91$; $p = 0.04$, Species within families: $F_{14,1} = 14.99$; $p = 0.20$; Random effects, Plot: $Z = 1.41$; $p = 0.16$, Family: $Z = 1.25$; $p = 0.21$).

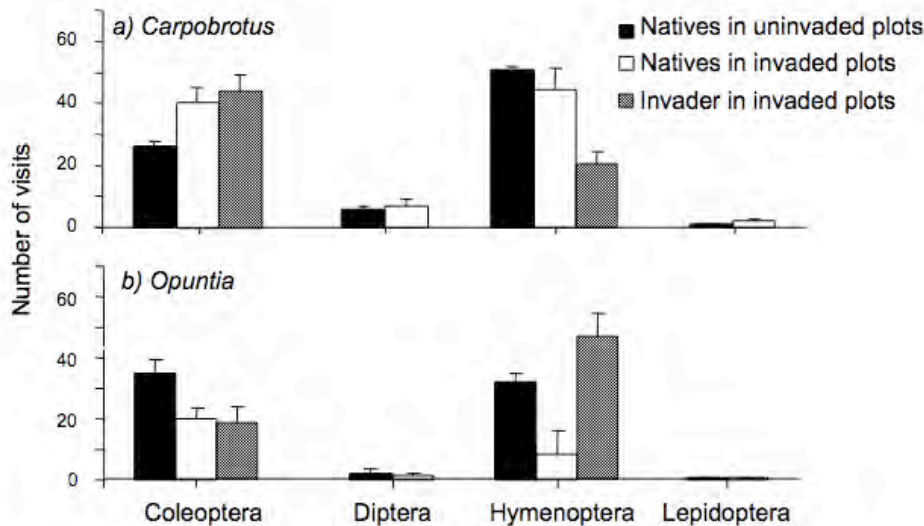


Figure 3.2: Total number of visits (mean + SE) for the different orders of insect pollinators observed in a) *Carpobrotus affine acinaciformis* and b) *Opuntia stricta* invaded and non-invaded plots. In invaded plots, visits to the plant invader and to native plant species are indicated

Plant-pollinator network in invaded versus non-invaded communities

The greater number of visits in *Carpobrotus*-invaded vs. non-invaded plots resulted in a marginal increase in the number of plant-pollinator interactions (67.67 ± 6.34 vs. 39.33 ± 16.37 , t test = 3.47 , $p = 0.07$). Nevertheless, paired *Carpobrotus*-invaded and non-invaded plots shared $57.66 \pm 8.01\%$ species interactions. Invaded plots had marginally more exclusive interactions than non-invaded plots (t test = 3.46 , $p = 0.07$). Most of the exclusive interactions, almost 70% in invaded plots, involved native plants. In fact, pollinators as a whole interacted with more plant species in *Carpobrotus*-invaded plots than in non-invaded plots (invaded: 2.33 ± 0.09 interactions/pollinator species, non-invaded: 1.94 ± 0.07 interactions/pollinator species; t test = 4.64 , $p = 0.04$). *Opuntia*-invaded and non-invaded plots shared even fewer common interactions ($31.43 \pm 3.56\%$). There were no significant differences in the total number of exclusive interactions



between invaded and non-invaded plots (t test = 0.24, $p = 0.4$). However, almost 50% of the exclusive interactions in invaded plots were due to interactions with *Opuntia* (Figure 3.3).

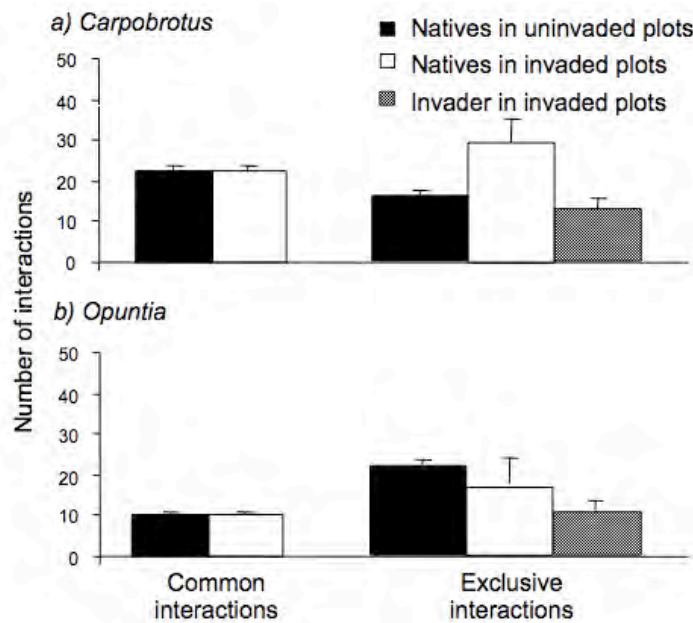


Figure 3.3: Mean (+ SE) number of common interactions and exclusive plant-pollinator interactions in a) *Carpobrotus affine acinaciformis* and b) *Opuntia stricta* invaded and non-invaded plots. In invaded plots, the exclusive interactions to the plant invader and to native plant species are indicated

The two dimensional representation of the non-metric multidimensional scaling (MDS) presents a stress of 0.001 for *Carpobrotus* and 0.05 for *Opuntia*, showing a high correspondence with the input data (similarities of the interaction identities). *Carpobrotus*-invaded plots were distinctly separated from non-invaded plots (Figure 3.4) and tended to cluster together along dimension 2. In two out of three sites, interaction identities differed significantly between paired native versus non-native plots (Wilcoxon test, site-BAT: $Z = 5.08$; $p < 0.0001$ and site-MED: $Z = 3.86$; $p < 0.001$). In contrast, although *Opuntia*-invaded plots also showed a consistent segregation from non-invaded plots along dimension 2 of the MDS, these differences were not significant in the identity of interactions (Wilcoxon test, $p > 0.1$ in all sites).



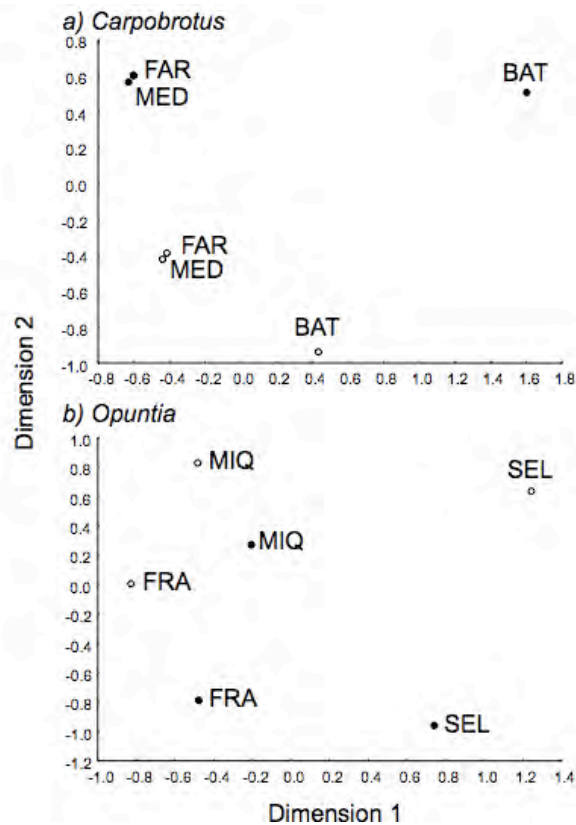


Figure 3.4: Representation of similarities of the plant-pollinator identity interactions in a) *Carpobrotus affine acinaciformis* and b) *Opuntia stricta* non-invaded (close circles) and invaded (open circles) plots, generated in two dimensions by multidimensional scaling analysis (MDS). See Table 1 for study site names and characteristics

The structure of the plant-pollinator network was significantly nested ($p < 0.05$) in half of the plots when comparing with the benchmark of 1000 random matrices for each plot with the Null Model 2 (Table 3.2). All *Opuntia*-invaded plots were significantly more nested than expected at random outcome, but none of the non-invaded plots were (Table 3.2). Nestedness did not differ between invaded and non-invaded plots for *Carpobrotus* (non-invaded vs invaded: 0.82 ± 0.002 vs. 0.77 ± 0.001 , $t = 1.34$, $p = 0.3$). However, *Opuntia*-invaded plots were marginally more nested than non-invaded plots (0.83 ± 0.0007 vs. 0.73 ± 0.0002 , $t = -3.49$, $p = 0.07$).

The strength of each native plant species was not significantly different between invaded and non-invaded plots (t test: $p > 0.05$ in all plots). The invasive plants had high strength values in all plots



and on average these were significantly higher than the strength value of native plant species (Table 3.2).

Table 3.2: Plant-pollination network parameters for *Carpobrotus affine acinaciformis* and *Opuntia stricta* invaded (_i) and non-invaded (_ni) plots. Strength values are for the invader and for native plants (mean \pm SE) in invaded and non-invaded plots. The normalized Z value and the associated probability (PZ) for the invader strength to be higher than natives are given. The temperature (T), nestedness (N) and the associated probability for T (PT) to occur by random has been tested using Null Model 2 according to Bascompte et al. (2003).

Site	Species	Strength	Z-value	PZ	T	N	PT
BAT	<i>Carpobrotus</i>	11,21					
	Natives_ni	3.4 \pm 0.74	2,70	0,007	13,29	0,87	0,1
	Natives_i	2.1 \pm 1.06	3,87	0,0001	23,07	0,77	0,001
MED	<i>Carpobrotus</i>	4,12					
	Natives_ni	1.93 \pm 0.60	1,21	0,22	16,53	0,83	0,05
	Natives_i	2.84 \pm 0.87	0,50	0,62	17,29	0,83	0,01
FAR	<i>Carpobrotus</i>	5,01					
	Natives_ni	2.41 \pm 0.73	1,18	0,23	26,52	0,73	0,2
	Natives_i	1.85 \pm 0.40	2,61	0,009	27,75	0,72	0,1
SEL	<i>Opuntia</i>	7,76					
	Natives_ni	2.12 \pm 0.79	2,51	0,012	29,38	0,71	0,2
	Natives_i	2.15 \pm 0.98	2,01	0,044	14,96	0,85	0,01
MIQ	<i>Opuntia</i>	10,28					
	Natives_ni	2.97 \pm 1.13	2,63	0,0085	25,53	0,74	0,08
	Natives_i	1.78 \pm 0.90	3,84	0,0001	20,91	0,79	0,01
FRA	<i>Opuntia</i>	7,10					
	Natives_ni	3.0 \pm 1.16	1,24	0,215	27,32	0,73	0,1
	Natives_i	2.1 \pm 0.74	2,42	0,015	14,29	0,86	0,01



Discussion

Our work, along with that of Lopezaraiza-Mikel et al. (2007), is unique in empirically testing how an invasive plant species can affect the plant-pollinator networks. While Lopezaraiza-Mikel et al. (2007) looked at the effect of a zygomorphic invasive plant; we investigated two actinomorphic species with different floral phenologies. The two invaders we studied had larger flowers than any of the co-occurring native species and attracted significantly more pollinator species than any native. However, the consequences for plant-pollinator networks differed between species. *Carpobrotus*-invaded plots received a larger number of visits, mainly from Coleoptera, resulting in an increasing number of plant-pollinator interactions. Although *Opuntia*-invaded plots received a similar number of visits as non-invaded plots, most visits were to the invasive species, thus reducing the visits to native plants. We examined the consequences of the presence of the two invaders from the perspective of the native plants, the pollinators, and the plant-pollinator network as a whole.

Implications for the native plants

In general, pollinator limitation does not appear to be a major barrier for the spread of introduced plants (Richardson et al. 2000). Many invasive plants are pollination generalists, with attractive nectar and pollen-rich flowers that constitute an easy resource (Campbell 1989). In our study, *Carpobrotus* received more visits than any native species; still, the average number of interactions (3-4 pollinator species/plant species) was within the range reported by Olesen et al. (2002) for oceanic islands. In invaded plots, the general trend was for an increased visitation rate to most native plant species. Only a few specialized species with zygomorphic flowers, such as Labiatae (*Lavandula stoechas*) and Papilionaceae (*Lathyrus clymenum*) received fewer visits in invaded than in non-invaded plots. These results seem to contradict the “floral market” hypothesis (Chittka and Schürkens 2001), which suggests that animals choose between products (plant species) on the basis of their quality (nectar and pollen). According to this hypothesis, resource-rich invasive plants with higher reward levels might receive higher visitation rates, thus decreasing visitation rates to the natives. Instead, *Carpobrotus* appears to have a facilitative effect on the



visitation to natives. Similar observations were made by Fleishman et al. (2005), who found that the higher the total amount of resources available in the community, the more pollinators are attracted to all plants. Whether this enhanced pollinator visitation translates to a positive effect on the native species seed set is not known. In these circumstances the pollen transport web might be dominated by the invader as found in Lopezaraiza-Mikel et al. (2007), and invasive pollen could be deposited on native stigmas interfering with fecundity. Preliminary results show that *Carpobrotus* pollen is indeed transported on pollinator's bodies, but few pollen grains reach native stigmas suggesting that the ultimate impact on natives is low (Bartomeus et al. Submitted; see chapter 4).

We expected *Carpobrotus* to have a higher impact on competition for pollinators than *Opuntia* because there were more species with flowering phenologies overlapping with *Carpobrotus* than with *Opuntia*. However, we found the contrary: although if *Opuntia* overlaps with late spring flowering native species, we found that it tended to monopolize the available pollinators, depriving native plant species of their service. This result suggests that the “floral market” hypothesis best fits situations where resources are scarce for pollinators. Overall, assuming that visitation rates are good surrogates for pollination efficiency (Vázquez et al. 2005; Sahli and Conner 2006), these two invasive plants could have contrasting effects on native plant reproduction: enhancement by *Carpobrotus*, but reduction by *Opuntia*.

Implications for the pollinator community

Although pollinator diversity in Mediterranean communities is not well known (Petanidou and Ellis 1993), this region is a well-established hotspot of bee diversity and speciation (Michener 2000). Despite current concern about the homogenization of pollinator diversity (Kearns et al. 1998; Steffan-Dewenter et al. 2005), the two invasive plants we studied did not reduce the diversity of pollinators. This may be a transient pattern, because our study was conducted in the early stages of invasion, and so native plant diversity and composition are only slightly affected. Given that pollinators tend to show a high degree of floral constancy (Kevan and Baker 1983), in sites at more advanced stages of invasion, in which native plant diversity has been reduced



(Lockwood and McKinney 2001; Vilà et al. 2006), pollinator diversity is more likely to be affected (Steffan-Dewenter and Tschardt 2001). Additionally, we do not know the effects that the invaders might have on pollinator fitness and behavior, such as altering nesting sites (Graves and Shapiro 2003). To date, evidence for the impact of invasive plants on native pollinator population size and dynamics remain speculative (Traveset and Richardson 2006).

In *Carpobrotus* invaded plots there were more visits by Coleoptera than in non-invaded plots. Coleoptera is a very generalist group of pollinators and little is known about their importance in Mediterranean plant pollination. Honey bees, which are maintained for honey production in several places in the Cap de Creus area, were not especially abundant neither in the *Carpobrotus*-invaded plots nor on *Carpobrotus* flowers (only $1.4 \pm 0.1\%$ of total visits). They were nonetheless present, visiting principally Labiatae flowers such as *Lavandula stoechas* and *Rosmarinus officinalis*. In *Opuntia*-invaded plots the presence of honey bees was high, especially on *Opuntia* flowers ($11.1 \pm 3.7\%$ of total visits). In our study super-generalist pollinators are not the main pollinators supporting invasive plants (Olesen et al. 2002).

In *Opuntia* invaded networks, *Xilocopa violacea* (usually considered a generalist pollinator (Westrich 1990)) visited *Opuntia* flowers exclusively. The absence of *X. violacea* from non-invaded networks confirms that insects can easily learn to choose and exploit new resources (Dukas and Bernays 2001; Weiss and Papaj 2003) and that, depending on the scale of observation, interactions that appear to be specialized can be interpreted as facultative or local specializations. Unraveling the pollination ecology of invasive plants in introduced and native ranges is a future challenge. A study on this aspect has shown that although the identity of pollinators visiting the invader might differ between introduced and native habitats, flower visitation rates might not be significantly different at home than abroad (Stout et al. 2006).



Implications for the plant-pollinator network

Invasion can also affect the identity of plant-pollination interactions. In *Carpobrotus* paired sites, the similarity of interaction scenarios reflected on the MDS showed few differences within non-invaded plots, especially between those with a similar floral composition, and a large separation with their paired invaded plots. In contrast, for *Opuntia* differences between invaded and non-invaded sites were not significant.

Generalization among plants and pollinators is a common pattern in mutualistic interactions, with strict specialists (i.e., one-to-one interaction) being the exception rather than the norm (Waser et al. 1996; Vázquez and Aizen 2004). In addition, asymmetric specialization predominates as reflected by the nestedness of the plant-pollinator networks (Bascompte et al. 2003). Our results indicate that although the nested plant-pollinator structure is very stable (Bascompte et al. 2003; Jordano et al. 2003) it can be modified with introduced species that have not coevolved in the system. Nestedness increased in *Opuntia* plots, probably because while no important links disappeared, invasive plants interacted with many pollinators, attaining a central role in the plant-pollinator network. Furthermore, the high strength value and the central role invasive species played in the plant-pollinator networks suggest that invasion could have long-term consequences, and the dependence of pollinators on the invader may not be reversible. Manipulation experiments are needed to test this prediction.

Conclusions

Both *Carpobrotus affinis acinaciformis* and *Opuntia stricta* are insect-pollinated invasive plant species that in our study behaved as super-generalists, occupying a central role in the community and reducing the protagonism of pollinator interactions with native species. However, they had contrasting effects on the relationships between plant and pollinators. Because of these invasive species attract a wide diversity and abundance of pollinators, the spatial and temporal scale at which this attraction operates can result in either positive or negative effects on the visitation to native plants. In our study, visitation rates to native plants were enhanced in plots that had been



invaded by *Carpobrotus* and reduced by plots invaded by *Opuntia*. *Carpobrotus* attracted pollinators which increased visitation to natives, whereas *Opuntia* diverted pollinators away from the native species in the community. This altered the structure of the plant-pollinator networks and resulted in an increase of nestedness in *Opuntia* invaded plots.

Invasive plants are considered to be the third major cause of pollinator diversity loss through changes in native flora (Kearns et al. 1998). However, few studies have tested this hypothesis except in agroecosystems (but see Steffan-Dewenter and Tschardt 2001). We envision that the outcomes of biological invasions on plant-pollinator interactions are context specific, and will depend on the invader identity and abundance, the overlap in phenology of the invader and the native plants in a local community, the flower similarity between the invader and native species as well as the indirect effects of the invader on native plant composition and diversity. For example, Vázquez et al. (2007), found that a large proportion of the variation in the observed plant-pollinator structure has been explained by differences in species abundance. Consequently, the impact of an invader will depend not only on qualitative traits but also on their dominance in the community. More experimental studies in different invasion scenarios are needed to disentangle the mechanisms involved in the impact of invaders on plant-pollinator networks.



High invasive pollen transfer, yet low deposition on native stigmas in a *Carpobrotus*-invaded community³

Abstract

Invasive plants are potential agents of disruption in plant-pollinator interactions. They may affect pollinator visitation rates to native plants and modify the plant-pollinator interaction network. However, there is little information about the extent to which invasive pollen is incorporated into the pollination network and about the rates of invasive pollen deposition on the stigmas of native plants. We tested the degree of pollinator sharing between the invasive plant *Carpobrotus affine acinaciformis* and the main co-flowering native plants in a Mediterranean shrubland. We identified pollen loads from the bodies of the ten most common pollinator species and stigmatic pollen deposition in the five most common native plant species. We found that pollinators visited *Carpobrotus* extensively. Seventy three percent of pollinator specimens collected on native plants carried *Carpobrotus* pollen. On average 23 % of the pollen on the bodies of pollinators visiting native plants was *Carpobrotus*. However, most of the pollen found on the body of pollinators belonged to the species on which they were collected. Similarly, most pollen on native plant stigmas was conspecific. Invasive pollen was present on native plant stigmas, but in low quantity. *Carpobrotus* is highly integrated in the pollen transport network. However, Mediterranean plant-pollinator communities seem to be sufficiently robust to withstand the direct effects of *Carpobrotus* on native plant pollination.

Introduction

Biological invasions caused by the intentional or accidental introduction of alien species are threatening the conservation of biodiversity through the local displacement of native species, changes in community structure and the modification of ecosystem function (Vitousek, 1994;

³ This chapter is in review in *Annals of Botany*. Bartomeus, Bosch, Vilà (in review)



Enserink et al., 1999). It has long been established that alien plants can interfere with native plants through direct competition for abiotic resources (i.e. soil nutrients, water, space and light) (Levine et al., 2003). In addition, biological invasions are increasingly viewed as potential agents to disrupt mutualistic interactions (Richardson et al., 2000; Mitchell et al., 2006; Traveset and Richardson, 2006) possibly resulting in changes in pollen transfer dynamics and subsequent plant reproductive success (Bjerknes et al., 2007).

Whether an entomophilous invasive plant facilitates or competes for pollinators and ultimately for pollen, depends on how pollinators respond to the temporal and spatial changes in resource availability (Knight et al., 2005), as well as the interference that invasive pollen may cause on native stigmas. Invasive plant species may change pollination patterns in many ways, such as through the decline of certain pollinator species, the disappearance of certain plant-pollinator interactions or the increase in exotic pollinators (Morales and Aizen, 2002; Olesen et al., 2002; Lopezaraiza-Mikel et al., 2007; Bartomeus et al., 2008). These changes in the pollinator community may result in increased or decreased visitation rates to native species (Chittka and Schürkens, 2001; Brown et al., 2002; Moragues and Traveset, 2005). Changes in visitation rates may also modify pollen transfer patterns from pollinators to stigmas. Low conspecific pollen and high invasive pollen deposition on native species could decrease the native plant seed set (Chittka and Schürkens, 2001; Brown et al., 2002; Moragues and Traveset, 2005; Larson et al., 2006).

However, studies describing the events underlying potential competition for pollen between invasive and native plant species are scarce (Knight et al., 2005). The effects of specific invasions appear to be context-specific and thus remain difficult to predict, given the complexity of the structure of plant-pollinator interactions (Bascompte et al., 2003; Bascompte and Jordano, 2007; Blüthgen et al., 2007). Competition for pollen requires (a) pollinator sharing between alien and native plants, (b) alien pollen transfer to the body of pollinators, (c) substantial alien pollen deposition on the stigmas of native plants, and (d) chemical or mechanical interference of alien pollen with native pollen.



In this study we analyse the potential competition for pollen between an invader plant with large, pollen rich flowers, *Carpobrotus affine acinaciformis* (*Carpobrotus* hereafter), and the main co-flowering native plants in a Mediterranean shrubland. Previous studies have shown that *Carpobrotus* could facilitate pollinator visitation to some plants, but compete with others (Moragues and Traveset, 2005). In a previous study we analysed the plant-pollinator network, showing that invaded communities attracted more pollinators, especially beetles, than non-invaded communities (Bartomeus et al., 2008; see chapter 3). Thus, *Carpobrotus* acted as a “magnet” species, like *Impatiens glandulifera* was coined in the UK (Lopezaraiza-Mikel et al., 2007). However, due to its profuse pollen production per flower, *Carpobrotus* could also potentially alter the network of pollen distribution in the community. In this study we measured pollinator visitation rates to *Carpobrotus* and coexisting native plant species, pollinator pollen loads, and pollen deposition on the stigmas of native plants. We asked the following questions: 1) What is the extent of pollinator sharing between native and invader species? 2) Do pollinators carry invasive pollen and are there differences among pollinator species in this regard? 3) Do pollinators carry more invasive pollen compared to heterospecific pollen from other native species? 4) Do pollinators visiting native plants carry invasive pollen? 5) Is invasive pollen deposited on native stigmas? *Carpobrotus* flowers produce large amounts of pollen, have an unspecialised morphology and pollen presentation, and are visited by a wide array of generalised pollinators (Bartomeus et al. 2008). Thus, our hypothesis is that *Carpobrotus* pollen is well integrated in the plant-pollination network and we expect significant pollen deposition on native stigmas.

Methods

Study area

The study area is located in a coastal Mediterranean shrubland invaded by *Carpobrotus* in the Natural Park of Cap de Creus (Catalonia - NE Spain). The community is dominated by several shrubs (*Pistacea lentiscus*, *Juniperus communis*, *Erica arborea*, *Lavandula stoechas*, *Rosmarinus officinalis*, and *Cistus* spp) and annual herbs (*Sonchus tenerrimus* and *Helianthemum guttatum*). *Carpobrotus* is the only invasive plant species. The area is characterised by cool, wet winters and



warm, dry summers. Mean temperatures of the coldest (January) and hottest (August) months in 2006 were 6°C and 23°C, respectively, and the annual precipitation was 450 mm (www.meteocat.com).

Invasive species studied

Carpobrotus (Aizoaceae) are crawling succulent chamephytes with fast clonal growth, that have been introduced from South Africa into almost all Mediterranean regions. In Spain, they were introduced for gardening and soil fixation at the beginning of the 20th century (Sanz-Elorza et al., 2006). Introgressive hybridization is common in *Carpobrotus* (Vilà et al., 2000). In the study area *Carpobrotus* are probably hybrids of *C. edulis* and *C. acinaciformis*. We follow Suehs et al. (2004) nomenclature and refer to our study plants as the hybrid complex *Carpobrotus affine acinaciformis*. Besides asexual reproduction, *Carpobrotus* has a generalist pollination system (Suehs et al., 2006) and a facultative outcrossing mating system (Vilà et al., 1998). *Carpobrotus* flowers are yellow to purple and measure 8–10 cm in diameter, being the largest flowers in the study community. In our study site, *Carpobrotus* flowers from early April to late May and accounts for 39% of the plant cover, with a peak density of 2.6 flowers/m², representing 9.75 % of floral units in the community.

Pollinator visitation rates

In spring 2005, we selected a representative 50 x 50 m invaded plot where we positioned two parallel 50-m permanent transects. We conducted the pollinator counts by observing 30 x 30 cm quadrats randomly positioned along the transects. This quadrat size allowed a close monitoring of plant-pollinator interactions. Each sampling day, we performed 6 observation quadrats per plant species to avoid oversampling of the most common species and to sample several individual plants per species. On every sampling day we recorded pollinator visits at each observation quadrat during four-minute periods. Sampling was conducted every two weeks (five times in total),



encompassing the entire flowering period of the invasive plant. Previous extensive surveys indicated that this sampling intensity was sufficient to characterize the pollinator community (Bartomeus et al., 2008). Sampling was conducted on non-windy, sunny days with temperatures higher than 15°C.

We focused on pollinator visits to *Carpobrotus* and the five most abundant flowering native plant species: *Cistus monspeliensis* (Cistaceae, 18 % cover), *Cistus salvifolius* (Cistaceae, 13 %), *Lavandula stoechas* (Lamiaceae, 7 %), *Cistus albidus* (Cistaceae, 6 %) and *Sonchus tenerrimus* (Asteraceae 5 %). Taken together, these five native species represented 49% of the plant cover and 66% of the flower abundance and received more than half of the pollinator visits to the community (Bartomeus, 2005). Data on the complete plant-pollinator network can be found in Bartomeus et al. (2008). We report visitation data on the ten most common pollinators (5 bee and 5 beetle species), accounting for 76% of the total visits recorded. All ten species visited both the invader and some of the native target species (Bartomeus, 2005). These taxa include two social bees: *Apis mellifera* (Apidae, 7.4 % visits) and *Bombus terrestris* (Apidae, 2.8 %), three solitary bees: *Andrena* sp. (Andrenidae, 13.8 %), *Anthidium sticticum* (Megachilidae, 8.1 %) and *Halictus gemmeus* (Halictidae, 1.9 %), and five beetles: *Oxythyrea funesta* (Scarabaeidae, 8.8 %), *Cryptocephalus* sp. (Chrysomelidae, 2.7 %), *Mordella* sp. (Mordelidae, 5.2 %), *Oedemera* spp. (including *O. flavipes*, *O. lurida* and *O. nobilis*; Oedemeridae, 7 %), and *Psilothrix* sp. (Dasytidae, 8 %). We used the Chi-square test to compare the visitation frequency of beetles and bees to *Carpobrotus* versus native species.

Pollinator pollen loads

In spring 2006, pollinators were collected throughout the *Carpobrotus* flowering period. For each plant-pollinator interaction observed we caught at least 15 pollinator individuals. In total, 474 pollinators were collected (298 on native plant species and 176 on *Carpobrotus*). All the native plants on which pollinators were collected were at a maximum of 5 m from a flowering *Carpobrotus*. To avoid pollen contamination among specimens, pollinators were caught in



individual, clean vials with cotton and a few drops of ethyl acetate. Two pollen samples were later obtained from each individual by gently rubbing small pieces of fuchsine-stained gelatine on their bodies (Kearns and Inouye, 1993). One pollen sample was taken from the ventral part and the other from the dorsal part of the pollinator. Pollen samples were mounted on microscope slides, and all pollen grains were identified and counted at 400 X magnification. Pollen identification was based on a reference collection of the main native species of the study area (Photo 4.1).

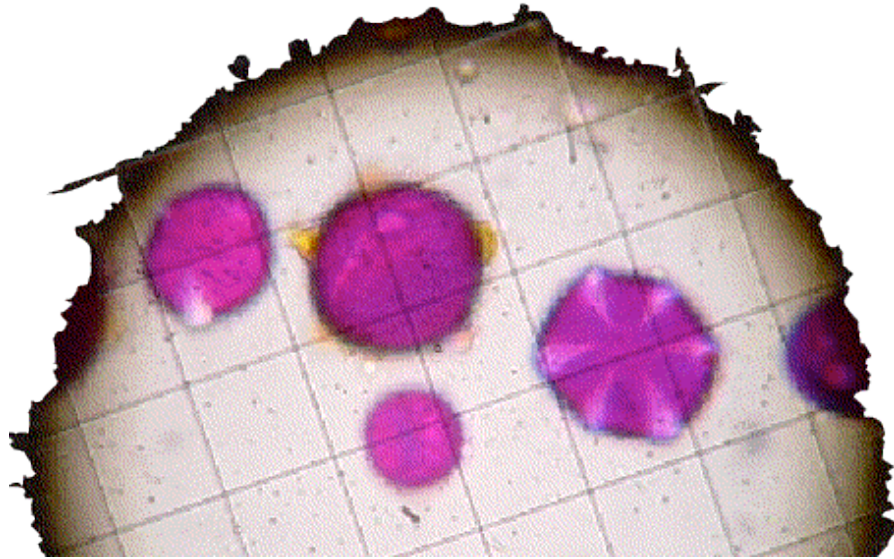


Photo 4.1: Pollen image of 4 different species. On the upper part, from right to left, we see *Carpobrotus*, *Cistus* and *Lavandula* pollen grains and centered in the bottom a *Vicia* pollen grain. The diameter of the squares is 0.03 mm.

This method did not allow us to accurately quantify pollinator pollen loads, but, because the same sampling effort was applied to each individual, we used the number of pollen grains in the samples as an estimator of pollen load. We grouped identified pollen grains into three categories: conspecific (pollen from the plant species on which the pollinator was caught), heterospecific (pollen from other native plant species) and invasive (*Carpobrotus*) pollen.

We compared differences between pollinator taxa in pollen species richness and in pollen loads. To assess the degree of incorporation of invasive pollen into the pollination network, we compared differences in the percentage of *Carpobrotus* pollen loads between pollinator taxa. Specimens caught on native plants were analysed separately from specimens caught on *Carpobrotus*. For each pollinator taxa we compared the percentage of invasive pollen carried by individuals collected on



Carpobrotus and by individuals collected on native species. Differences in conspecific pollen loads across pollinator taxa visiting native plants were also tested. For each pollinator taxa, the percentage of the dominant heterospecific native pollen and invasive pollen was compared. Finally, to describe the general pattern of pollinator pollen loads when visiting different native plant species, we compared whether there were differences in conspecific pollen loads across visited plants, and whether there were differences in invasive pollen loads across native species. One way ANOVAs were used to test differences between pollinator taxa and plant species. For all ANOVAs, posthoc Fisher tests were conducted to assess pair-wise differences. Contrasts within pollinator taxa were conducted with t-tests.

Stigma pollen loads

We collected 30 stigmas (one from each of 30 individuals) per plant species along the plant flowering period. Flower buds were marked, and stigmas were collected on the day after the maximum receptivity according to the literature (Bosch 1992 for *Cistus* spp, Devesa et al., 1986 for *L. stoechas*) and personal observations. Stigmas were squashed on microscope slides with fuchsine-stained gelatine and identified at 400 X. Sometimes, pollen grains were clumped or masked by stigma tissue, so that accurate pollen counts were not feasible. Thus, for each pollen type (conspecific, heterospecific, invasive) we established five abundance categories: absent (no pollen grains), present (only one pollen grain); low (< 20 % of the total pollen grains representing approximately 20-40 pollen grains); moderate (20-70 %) and high (> 70%). We compared differences in frequency of pollen abundance categories on stigmas between heterospecific and invasive pollen with Chi-Square tests. A different Chi-Square test was used for each abundance category.





Table 4.1: Percentage of visits by the 10 most abundant pollinator species to the invader *Carpobrotus* and the five most abundant native plant species.

	Bees					Beetles				
	<i>Andrena sp.</i>	<i>Anthidium sticticum</i>	<i>Apis mellifera</i>	<i>Bombus terrestris</i>	<i>Halictus gemmeus</i>	<i>Cryptocephalus sp.</i>	<i>Mordella sp.</i>	<i>Oedemera spp.</i>	<i>Oxythyrea funesta</i>	<i>Psilothrix sp.</i>
<i>Carpobrotus aff. acinaciformis</i>	5,41	11,36	17,50	93,33	20,00	33,33	61,54	32,91	38,46	56,41
<i>Cistus albidus</i>	13,51	11,36	5,00	6,67				8,86	7,69	
<i>Cistus monspeliensis</i>	22,97				20,00			32,91	25,00	2,56
<i>Cistus salvifolius</i>	21,62		5,00				23,08	10,13	23,08	17,95
<i>Lavandula stoechas</i>	4,05	68,18	37,50		60,00				1,92	
<i>Sonchus tenerrimus</i>	31,08	2,27				66,67	15,38	15,19	1,92	23,08

Results

Visitation rates

A total of 323 (51% of the total survey on the community) visits were recorded to target native plants and 172 (35% of the total) to *Carpobrotus*. All 10 target pollinator taxa visited *Carpobrotus* and at least one of the target native species (Table 4.1). Bee and beetle visitation frequency differed between *Carpobrotus* and native plants (Chi-square = 79.03, $p < 0.0001$). Bees were more often recorded on native species than on *Carpobrotus*, except for *Bombus terrestris* that visited *Carpobrotus* flowers almost exclusively. In contrast, beetles tended to favour *Carpobrotus* over natives, except for *Cryptocephalus* sp., that visited mostly *Sonchus tenerrimus*.

Pollinator pollen loads

We identified a total of 139,063 pollen grains from the bodies of the collected pollinators. The average number of pollen species (including species other than our five target species and *Carpobrotus*) per individual pollinators was 3.17 and ranged from one to 8 with a mode of 3. Pollen loads and pollen species richness differed among pollinator species ($F(9, 464) = 11.27$, $p < 0.0001$; $F(9, 464) = 26.32$, $p < 0.0001$, respectively; Table 4.2).

Seventy-three percent of the pollinators collected on native plants carried *Carpobrotus* pollen grains, and, on average, pollinators visiting native plants carried 23.38 ± 4.02 % (mean \pm S.E.) invasive pollen. However, there were significant differences among pollinator species in the percentage of invasive pollen loads ($F(9, 288) = 7.73$, $p < 0.0001$, Figure 4.1; only individuals caught on native plants included). The beetles *Oxythyrea funesta*, *Psilothrix* sp. and the solitary bee *Anthidium sticticum* were the pollinators that carried more *Carpobrotus* pollen (Table 4.2). In general, when visiting native plants, beetles carried a higher proportion of *Carpobrotus* pollen than bees (33% vs 19%, t -test = 3.75, $p = 0.002$).





Table 4.2: Mean percentage (\pm SE) of conspecific, heterospecific native and invasive (*Carpobrotus*) pollen, and total number of pollen species carried by bees and beetles collected on the 5 most abundant native plant species (see Table 1) at a *Carpobrotus* invaded site. The average (\pm SE) number of pollen grains counted for each pollinator species is also provided. Different letters indicate significant differences within a column.

Pollinators	Order	N° of pollen species	Total pollen	Conspecific	Heterospecific	Dominant		
						heterospecific	Invasive	
<i>Andrena sp.</i>	Bee	2.84 \pm 0.10 b	461.42 \pm 91.46 ab	60.76 \pm 4.93 a	25.25 \pm 4.63	24.51 \pm 4.44	13.99 \pm 1.80	a
<i>Anthidium sticticum</i>	Bee	6.57 \pm 0.39 c	181.64 \pm 24.43 b	18.76 \pm 7.73 c	41.70 \pm 6.72	25.06 \pm 4.74	39.54 \pm 10.57	b
<i>Apis mellifera</i>	Bee	3.08 \pm 0.24 ab	716.92 \pm 118.54 a	58.67 \pm 9.16 a	23.68 \pm 5.23	14.48 \pm 2.36	17.65 \pm 4.72	a
<i>Bombus terrestris</i>	Bee	4.33 \pm 0.54 a	889.92 \pm 170.36 a	69.61 \pm 6.21 a	19.32 \pm 5.36	12.82 \pm 4.32	11.07 \pm 4.67	a
<i>Halictus gemmeus</i>	Bee	2.94 \pm 0.22 b	376.19 \pm 54.16 bc	49.88 \pm 6.30 ab	28.88 \pm 5.85	23.07 \pm 4.47	21.24 \pm 4.16	a
<i>Cryptocephalus sp.</i>	Beetle	2.50 \pm 0.27 b	49.60 \pm 14.70 b	78.60 \pm 6.20 a	10.31 \pm 5.08	9.14 \pm 4.35	11.07 \pm 3.50	a
<i>Mordella sp.</i>	Beetle	1.25 \pm 0.63 b	4.75 \pm 2.06 b	67.50 \pm 23.58 a	17.53 \pm 2.50	10.33 \pm 3.58	14.07 \pm 4.57	a
<i>Oxythyrea funesta</i>	Beetle	3.69 \pm 0.12 ab	340.46 \pm 53.19 bc	39.90 \pm 4.01 b	12.90 \pm 2.37	10.20 \pm 1.78	47.19 \pm 5.64	b
<i>Oedemera spp.</i>	Beetle	2.33 \pm 0.09 b	47.18 \pm 6.74 b	57.48 \pm 4.21 a	18.27 \pm 3.10	15.50 \pm 2.84	24.20 \pm 2.85	a
<i>Psilothrix sp.</i>	Beetle	2.19 \pm 0.15 b	26.79 \pm 7.82 b	53.69 \pm 6.94 ab	12.46 \pm 2.68	10.21 \pm 2.24	33.83 \pm 6.91	ab
Mean		3.02 \pm 0.47	309.49 \pm 97.57	55.48 \pm 5.32	21.03 \pm 2.96	16.64 \pm 1.59	23.38 \pm 4.02	

Pollinators visiting *Carpobrotus* carried mostly *Carpobrotus* pollen, but there were significant differences among pollinator species ($F(9, 170) = 3.96, p < 0.001$, Figure 4.1), with *A. mellifera*, *B. terrestris* and *Psilothrix sp.* scoring highest. As expected, pollinators collected on *Carpobrotus* carried more *Carpobrotus* pollen than pollinators collected on native plant species (all t-test $p < 0.05$), except for *Oxythyrea funesta* for which differences were not significant (t-test = 1.99, $df = 14, p = 0.07$).

Overall, the percentage of conspecific pollen (55.49 ± 5.32) on the bodies of the pollinators visiting native plants was higher than the percentage of heterospecific (21.03 ± 2.96) and invasive pollen (23.38 ± 4.02). There were significant differences among pollinators in conspecific pollen loads ($F(9, 288) = 4.17, p < 0.001$) which ranged from 19% in *Anthidium sticticum* to 79% in *Cryptocephalus sp.* (Table 4.2).

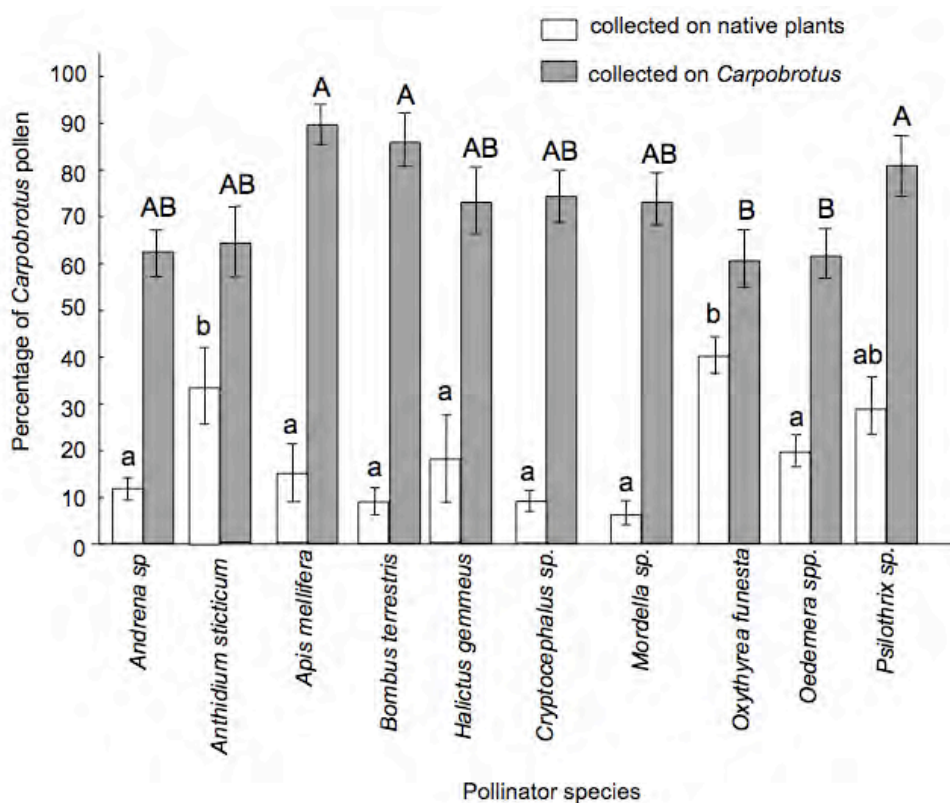


Figure 3.1: Percentage (mean \pm S.E.) of *Carpobrotus* pollen loads from the bodies of the 10 most abundant pollinator species collected on native plant species (white bar) and on the invader *Carpobrotus* (black bar). Different letters indicate significant differences between pollinators for native species (lower case) and *Carpobrotus* (upper case).



For each pollinator species, we analyzed differences between the dominant heterospecific native pollen and *Carpobrotus* pollen loads. We found that while *Andrena* sp. and *Cryptocephalus* sp. carried more pollen of the dominant heterospecific species than invasive pollen (paired t-test = 2.00, df = 74, p = 0.05; t = 4.02, df = 14, p < 0.001, respectively), *Oxythya funesta* and *Psilothrix* sp., carried more invasive pollen than the dominant heterospecific native pollen (paired t-test = 4.31, df = 74, p < 0.001; t = 3.22, df = 49, p < 0.006, respectively). There were no significant differences for the other pollinator taxa (paired t-test, all p > 0.2).

There were significant differences in the percentage of conspecific pollen carried by pollinators depending on the plant species on which they were collected ($F(5, 464) = 20.91$, p < 0.001). *Sonchus tenerrimus* and *Carpobrotus* were the species whose pollinators carried a higher percentage of conspecific pollen. The percentage of invasive pollen loads carried by pollinators varied depending on the native species on which they were collected ($F(4, 288) = 12.06$, p < 0.001). Pollinators collected on *Sonchus tenerrimus* carried less invasive pollen than pollinators collected on the other plant species (Figure 4.2).

Stigma pollen loads

Almost all stigmas were fully covered with pollen. We found 2.13 ± 0.1 pollen species per stigma. Plant species differed in pollen species richness ($F(4, 145) = 14.99$, p < 0.001), ranging from 1.5 in *Lavandula stoechas* to 3.5 in *Cistus albidus*. For all species, conspecific pollen was the most common type. Heterospecific and invasive pollen were found on all plant species, but were never abundant.



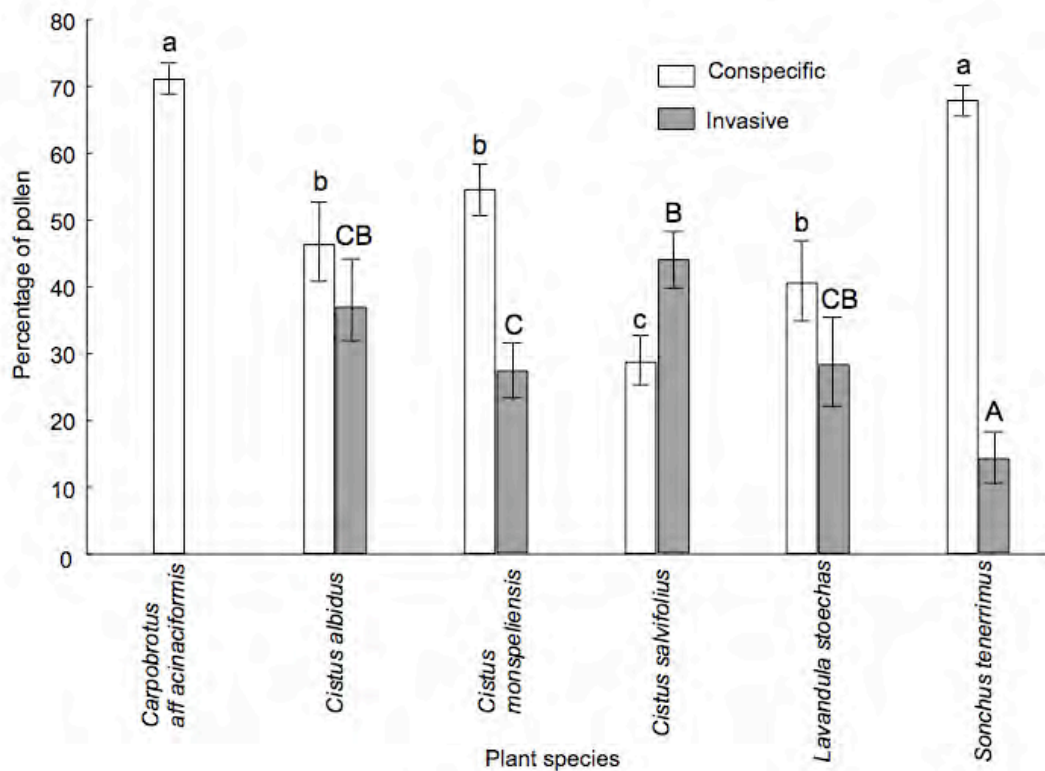


Figure 4.2: Percentage (mean \pm S.E.) of conspecific (white bar) and invasive pollen (black bar) loads on pollinators collected on different plant species. Different letters indicate significant differences between plant species for conspecific pollen (lower case) and invasive pollen (upper case).

Of the 150 native stigmas sampled, 36 % had invasive pollen. Taking into account all plant species, frequencies of heterospecific pollen abundance categories were different from frequencies of invasive pollen abundance categories (all χ^2 , $p < 0.0001$). Heterospecific pollen counts were mostly in the low and moderate abundance categories, whereas invasive pollen counts were mostly in the absence and presence categories. That is, *Carpobrotus* pollen was less abundant than total heterospecific pollen (Table 4.3).





Table 4.3: Mean (\pm S.E.) number of pollen species on the stigmas of the five most abundant plant species at an invaded *Carpobrotus* site, and percentage of stigmas with various abundance categories of conspecific, heterospecific native and invasive (*Carpobrotus*) pollen. Absent: No pollen; present: 1 pollen grain; low: Less than 20 % of the total pollen load; moderate: 20-70 % of the total pollen load; and high: More than 70% of the total pollen load.

Plant species	Pollen species	Conspecific					Heterospecific					Invasive				
		absent	present	low	moderate	high	absent	present	low	moderate	high	absent	present	low	moderate	high
<i>Cistus albidus</i>	3.6 \pm 0.29	0	0	0	0	100,0	0	10,00	75,0	15,0	0	45,0	0	45,0	10,0	0
<i>Cistus monspeliensis</i>	1.6 \pm 0.16	0	0	0	10,0	90,0	60,00	3,40	36,7	0	0	86,6	0	13,3	0	0
<i>Cistus salvifolius</i>	2.3 \pm 0.20	0	0	0	0	100,0	32,20	16,1	45,1	6,4	0	41,9	25,8	25,8	6,5	0
<i>Lavandula stoechas</i>	1.5 \pm 0.16	0	16,7	33,3	10,0	40,0	46,40	20,0	33,3	0	0	90,0	10,0	0	0	0
<i>Sonchus tenerrimus</i>	2.2 \pm 0.19	0	0	0	6,7	93,3	33,30	23,3	36,7	6,7	0	60,0	20,0	13,3	6,7	0
Total natives	2.2 \pm 0.20	0	3,3	6,7	5,3	84,7	34,38	14,6	45,4	5,6	0	64,7	11,2	19,5	4,6	0

Discussion

Carpobrotus flowers produce large amounts of readily accessible pollen and attract a wide range of pollinator species (Suehs et al., 2004). At our site, there was a substantial overlap in pollinators between *Carpobrotus* and the most abundant native plants in the community. *Carpobrotus* pollen was efficiently transferred to the bodies of all pollinators sampled, supporting the hypothesis that invasive pollen is well integrated in the plant-pollination network (Memmott and Waser, 2002). All ten pollinator species studied carried *Carpobrotus* pollen (albeit in low numbers) when collected on native plant species. However, stigma pollen loads contained mostly conspecific pollen and invasive pollen was only rarely found on native plant stigmas. Therefore, even if *Carpobrotus* is the most abundant plant species in the community, produces large amounts of pollen compared to native species, and shares generalist pollinators with the most abundant native plant species, the likelihood of invasive pollen interfering with conspecific native pollen appears to be low.

Narrow specialization is rare in plant-pollinator relationships (Jordano, 1987; Waser et al., 1996). At our site, all pollinators visited various plant species and carried more than one pollen species. Most pollinators carried pollen from 2 to 4 species, and the bee *Anthidium sticticum* carried the pollen of as many as 6 pollen species. However, both bees and beetles mainly carried pollen from the plant on which they were caught.

There were differences in pollen loads between pollinator species. This could be related to body size and morphology (presence of hairs) (Adler and Irwin, 2006). Bees, which are viewed as the most efficient pollinators (Proctor et al., 1996), carried more pollen than beetles and visited native flowers more frequently than invasive flowers. Among bees caught on native plants, *Carpobrotus* pollen was always less abundant than the dominant heterospecific pollen. Beetles accounted for a high proportion of visits, and some beetles (*Oxythyrea funesta*) carried large quantities of *Carpobrotus* pollen. Beetles are typically viewed as poor pollinators (Proctor et al., 1996), although in Mediterranean ecosystems they are very abundant floral visitors (Dafni et al., 1990; Bernhardt,



2000). However, beetles spend long periods of time on each flower they visit, and therefore visit much fewer flowers than bees (Bosch, 1992). We observed *O. funesta* and *Psilothrix* sp. spending the night inside closed *Carpobrotus* flowers. Individuals of these two beetle species caught on native plants carried more *Carpobrotus* pollen than any other heterospecific pollen and, overall, carried more *Carpobrotus* pollen than bees.

All stigmas were fully covered with pollen. As with pollinator pollen loads, stigmas had mostly conspecific pollen. Neither heterospecific nor invasive pollen were present in high proportions. Even *Cistus salvifolius*, whose pollinators carried more invasive than conspecific pollen, had stigmas thoroughly covered with conspecific pollen. Moreover, processes of chemical pollen interference between pollen of distantly-related genera are rare (Heslop-Harrison, 2000; Brown and Mitchell, 2001), suggesting that *Carpobrotus* does not have a strong impact on the seed set of coexisting native plants. This is supported by another study, where even an experimental addition of a mixture of *Carpobrotus* and conspecific pollen on the stigmas of native emasculated flowers caused no negative effect on the seed set (Moragues and Traveset, 2005).

Although we have not explored whether native plants are pollen-limited for their seed set, these findings suggest that invasive pollen does not interfere with native species' seed sets. These results support findings in the Balearic Islands, in which a very low deposition of *Carpobrotus* pollen on native stigmas was found (Moragues and Traveset, 2005). Moreover, the presence of *Carpobrotus* not only did not decrease visitation rates to native plants but even resulted in an increase in pollinator visitation to some native plants (Moragues and Traveset, 2005; Bartomeus et al., 2008). Therefore, and accounting that visitation rates are good surrogates for pollination contribution (that is, frequent pollinators usually make significant contributions to a plant's seed set; Vázquez et al., 2005; Sahli and Conner, 2006), it is not likely that *Carpobrotus* competes with native plants for pollination

Plant-pollinator interactions are generalized, with most plants receiving visits from several pollinators and most pollinators visiting several plants (Waser et al., 1996). This property



facilitates the integration of invasive plants into the pollination network (Memmott and Wasser, 2002; Lopezaraiza-Mikel et al., 2007), but at the same time appears to make plant-pollination networks robust and resilient to changes in pollinator and plant composition (Memmott et al., 2004). While plants and pollinators have probably co-evolved within generalised networks (Jordano, 1987; Jordano et al., 2003; Bascompte et al., 2006), they have developed effective mechanisms to ensure successful pollination (Knight et al., 2005; Blüthgen et al., 2007). For this reason, the incorporation of *Carpobrotus* species is unlikely to result in the collapse of the pollination network via competition for pollinators.

Carpobrotus grow so vigorously that competition for space and soil resources may be of greater importance to native plants (Vilà et al., 2006) than competition for pollinators. Even if *Carpobrotus* shares pollinators with native plant species and efficiently transfers its pollen to pollinators that have not coevolved with *Carpobrotus*, several mechanisms may contribute to prevent high levels of *Carpobrotus* pollen deposition on native plant stigmas. First, pollinator fidelity (floral constancy) is widely reported for most pollinator groups, including bees and beetles (de los Mozos and Medina, 1991; Goulson et al., 1997; Goulson and Wright, 1998; Gegeer and Thomson, 2004) and plays a very important role in conspecific pollen transfer. Second, due to differences in flower morphology and stigma position, pollinators carry different pollen species on different body parts (Ambruster et al. 1994); for example, disc-shaped flowers such as *Carpobrotus* or *Cistus* deposit their pollen on the ventral parts of pollinators, while labiate flowers, such as *Lavandula stoechas* deposit their pollen on the dorsal part of the pollinator's body. Third, there are differences among plant species in temporal pollen presentation; for example, *Carpobrotus* flowers close at night and open later in the day, whereas *Cistus* spp. anthesis occurs early in the morning and flowers lose their petals and close by early afternoon (Bosch 1992). Fourth, beetles have a low flower visitation rate, and even though they are abundant on flowers (including *Carpobrotus*), their contribution to pollination might be low (Bosch 1992). Low invasive pollen transfer from pollinators to native flower stigmas has also been reported in other systems, such as in areas invaded by *Impatiens glandulifera*, in which invader pollen dominates the pollen transport network, but its deposition on native stigmas is low (Lopezaraiza-Mikel et al., 2007).



In summary, invasive plants may negatively affect plant communities in many ways (Levine et al., 2003), and some invasive species have been found to have an impact on the pollination of native plants (Chittka and Schürkens, 2001; Brown et al., 2002). In our system, we found the invader to be highly integrated in the pollen transport network because all pollinators carried invasive pollen, but deposition of *Carpobrotus* pollen on stigmas of native plants was low. Together with another study (Moragues and Traveset, 2005) our results indicate that Mediterranean plant-pollinator communities are sufficiently robust to invasion by *Carpobrotus*.



Combined effects of *Impatiens glandulifera* invasion and landscape structure on native herb pollination⁴

Abstract

Habitat loss, land use intensification (i.e. agricultural land cover) and biological invasions are threatening pollinator communities, but combined effects of these factors on pollination have been rarely studied. Here, we tested the hypothesis that the invasive plant species *Impatiens glandulifera* outcompetes native plant communities for pollinators, attracting different densities of pollinators in agricultural landscapes than in natural landscapes. We selected 14 study sites along a gradient of increasing landscape complexity each with paired locally invaded or non-invaded riparian habitats. We performed standardised surveys of pollinators and established experimental plots by adding the native plant *Raphanus sativus* to assess the impact of *Impatiens* on visitation rates and seed set. Agricultural land use enhanced flower visitation rates of bumblebees, but in the presence of *Impatiens* this landscape effect was masked off by the high attraction of bumblebees to *Impatiens* stands independent from the structural complexity of the surrounding landscape. *Impatiens* was well integrated in the plant-pollinator network by frequent interactions with native pollinators, mainly bumblebees and received higher visitation rates than simultaneously flowering native riparian plants and the experimentally exposed *R. sativus*, but there were no significant differences on visitation rates to natives between invaded and non-invaded plots. Furthermore, *Impatiens* had not a significant effect on *R. sativus* seed set. We conclude that both, landscape and invasion, can affect the pollinator behaviour, and that plant invasion can modulate larger landscape effects on pollinators. However, both factors had not any effect on native plants and there are not evidences of competition for pollinators.

Introduction

Destruction of natural habitats, land use intensification and biological invasions are the most prominent components of global change influencing biodiversity in human-dominated temperate

⁴ This chapter has been submitted to Journal of Ecology. Bartomeus, Vilà and Steffan-Dewenter (Submitted)



ecosystems (Sala et al. 2000, Hobbs 2000). However, these components have been approached as single-factor conservation problems, rather than interacting factors (Vilà et al. 2006). To discriminate how these global change drivers influence species diversity and biological interactions a combination of approaches incorporating quantitative observational data and field experimentation is required (Kremen et al. 2007, Aizen 2007).

Pollinator loss is a major concern for conservation because pollination is essential for the reproduction of many wild plants and maintenance of genetic diversity (Burd 1994, Kearns et al. 1998, Steffan-Dewenter et al. 2005). Additionally, many agricultural crops depend on wild, unmanaged pollinator communities for crop pollination services (Klein et al. 2007). Parallel declines in pollinators and insect-pollinated plants have been reported in Britain and the Netherlands (Biesmeijer et al. 2006), presumably caused by habitat alteration and agricultural intensification.

Pollinator communities and plant-pollinator interactions are influenced by the landscape context such as land-cover type, successional age or habitat fragmentation (Steffan-Dewenter et al 2001, Aizen et al. 2002, Aguilar et al. 2006). For instance, local abundance and diversity of bees increase with increasing proportion of seminatural habitats in agricultural landscapes (Steffan-Dewenter et al. 2002). Moreover, each pollinator guild can be influenced by different factors acting at multiple spatial scales. For example, key resources for wild bees with small foraging distances are nesting sites and flowering plants provided by natural or seminatural habitats (Westrich 1996, Gathmann and Tscharrntke 2002). In contrast, social bees such as bumblebees that have broader foraging ranges benefit from the occurrence of mass flowering resources in agricultural fields at larger spatial scales (Westphal et al. 2003).

Biological invasions can also alter plant-pollinator interactions (Traveset and Richardson 2006; Bjerknes et al. 2007) but there are few empirical studies to draw general patterns. Plant invasions decrease or increase the number of visits to particular co-flowering native plants, depending on the invader and the specific context (Chittka and Schürkens 2001; Brown et al. 2002; Moragues and Traveset 2005; Larson et al. 2006). Further, even where the presence of the invader changes



visitation rates to native plants, this does not always reduce the seed set (Totland et al. 2006). Furthermore, the few existing studies analysing the effect of a plant invader on whole the plant-pollinator network have shown that the invader is very well integrated in the network (Memmott and Wasser 2002) and is sometimes acting as a magnet species (Lopezarriaza-Mikel et al. 2007) with potentially beneficial effects for pollination of native species (Bartomeus et al. 2008).

We are not aware of empirical studies that have investigated the combined effects of land use intensification and invasion on plant-pollinator networks. This goal is imperative to assess the magnitude of their impacts, to disentangle whether they interact additively or synergistically and also to justify mitigation actions to overcome their effects (Sala et al. 2000, Vane-Wright et al. 1991). We combined an observational and experimental approach along a landscape complexity gradient with paired invaded and non-invaded sites in Central-European riparian habitats to test how these two factors influence plant-pollinator interactions. We addressed the following hypothesis:

- I. The richness and abundance of different native pollinators guilds is dependent upon the landscape context.
- II. Invasive plants outcompete native plants for pollinators, thereby leading to reduced visitation rates and lower seed set.
- III. The invader can have a mass-flowering effect on pollinators attracting pollinators differentially in agricultural landscapes compared to natural landscapes.

Our study system focuses on the invasive plant *Impatiens glandulifera* (*Impatiens hereafter*), a noxious invader by reducing species diversity and outcompeting native flora (Beerling and Perrins 1993, Wade et al 1994, Hulme and Bremner 2006). First, we surveyed pollinator assemblages and flower visitation rates of the whole riparian plant community in invaded and non-invaded plots along a landscape complexity gradient. Second, we experimentally exposed individuals of the native plant *Raphanus sativus* in the same sites to also measure the impact of the invader on visitation rates and seed set. *Raphanus sativus* is visited by a wider pollinator spectrum than *Impatiens* (Conner et al. 1995). Therefore, our study differs from previous ones where the target



native species potentially competing with *Impatiens* were more specialised (Brown et al. 2002, Chittka and Schurkens 2001). We repeated the study before and during flowering of *Impatiens* to exclude phenological shifts of riparian plant communities and the surrounding crop areas as confounding factors.

Study species

The Indian balsam (*Impatiens*) is a widespread invasive riparian plant from the Himalayas. It was introduced in Europe as a garden ornamental plant and it has been naturalized since the early XX century. It has spread to most temperate communities of Europe, growing along river and stream banks, where it forms dense linear stands (Beerling 1993, Pyšek 2005). Usually, the Indian balsam is the tallest (up to 2 m) annual plant in the invaded area. It bears large inflorescences with purple zygomorphic flowers (3-4 cm long), it is protandrous and self-compatible, but because it produces a large amount of nectar and pollen, it receives constant visits, and consequently is frequently cross-pollinated (Valentine 1978). Attracted pollinators include several species of bumblebees, honeybees and wasps. *Impatiens* has a high impact on native plant species establishment, diversity and composition (Beerling and Perrins 1993, Hejda and Pyšek 2006). It can also alter water flow and cause increasing riverside erosion and flooding.

The annual *R. sativus* (Brassicaceae) was used as a target native plant species. *Raphanus sativus* is visited by a wide array of pollinators including honey bees, bumblebees, wild bees, syrphids and butterflies (Conner et al. 1995). The plant grows fast up to 1.5 m and flowers are numerous and self-incompatible (Ellstrand 1984, Young and Stanton 1990).

Study sites

The study was performed in Southern Lower Saxony, around the city of Göttingen, central Germany. The climate is temperate with a mean annual temperature of 8-8.5 °C and average



annual rainfall ranging from 600 to 700 mm (<http://www.wetterstation-goettingen.de/klimabericht.htm>). The study region is characterized by a highly agricultural landscape dominated by wheat, oilseed rape, other annual crops, grasslands and embedded patches of forests and grasslands.

In summer 2006, 14 landscape sites of 3000 m radii were selected within a 30x35 km area (Figure 5.1). The centre of each site was located at a river where the invader *Impatiens* was present. Along each riverside we placed two fixed 100 m long, 2 m wide transects. These two transects were placed at a distance of 100 m. One of these transects was invaded, and the other was non-invaded (Figure 5.2). The percentage ground cover of *Impatiens* in invaded transects ranged from 7% to 71% and the density of flowers from 6.2 to 45.5 flowers/m².



Figure 5.1: Land cover in a 3000 m radius area for two landscape sites near Göttingen (Germany). The centre of each site was located at a river where the invader *Impatiens glandulifera* was present. Agricultural fields, natural land cover (grassland + forest) and urbanized land uses are shown. The site to the left has 17% of natural land cover and the one on the right 71%.

The landscape context of each site was characterized by existing commercially available digital maps (ATKIS-DLM 25/1, 1991-1996; Landesvermessungsamt + Geobasisinformationen Niedersachsen, Hannover, Germany) using the Arcview GIS software in 3000, 1500 and 500 m radii from the centre of each site. We computed the composition of the landscapes as the percentage of natural (i.e. forests + grasslands), agricultural and urbanized land-cover, and river length. The mean area and perimeter of natural area patches were estimated as indicators of fragmentation and longitude of the ecotones, respectively. For most of the analysis only variables at the 3000 m radii were used.



The percentage of natural land cover within the 3000 m radii ranged from 12% to 66%. It was highly correlated with the percentage of agricultural fields ($r_2 > 0.90$; $p < 0.05$ for all radii). The mean (\pm SE) area (3.10 ± 0.2 ha) and perimeter (890.1 ± 27.9 m) of the natural patches was also highly correlated with the percentage of natural habitats (area: $r_2 = 0.80$, $p < 0.05$; perimeter: $r_2 = 0.70$, $p = 0.05$) and its variance was small (area: 3.10 ± 0.2 ha; perimeter: 890.1 ± 27.9 m). The river length ranged between 11.61 and 49.73 km and it was not correlated with the invader plant cover ($r_2 = 0.1$, $p = 0.67$).

Survey of the pollinator community

In each site we conducted three 30 minute transect walks (one per week) along the invaded and non-invaded 100 m transects. For each flowering plant we counted the number of floral units (Dicks et al. 2002) and the number and identity of all pollinator visits. Unknown pollinators were caught for later identification. In order to disentangle phenological differences from invasion patterns, sampling was done before *Impatiens* flowering (20 June - 15 July) and during its flowering peak (25 July - 20 August).

***Raphanus sativus* experiment**

We grew *R. sativus* plants in 4 l pots filled with commercial garden soil (Einheitserde T25) in outdoor conditions at the Göttingen University experimental garden facilities. This permitted us to compare plant treatments with standardized soil conditions and the same seeds origins. Just before the plants started flowering we placed three pots of *R. sativus* within each invaded and non-invaded transect (a total of 84 pots). Plants were watered as needed in both outdoor conditions and also in the field.



Once a week during three consecutive weeks we performed 15 minute observations recording the number and identity of all pollinator visits and counting the total number of flowers. As in the above described community surveys, pollinators that could not be identified in the field were caught for later identification. As before, we repeated this protocol twice, i.e. before and during flowering of *Impatiens*. Overall, data are based on observations of 168 pots in 14 landscape sites.

To compare patterns of pollination visitation to *R. sativus* with *Impatiens* we performed three 15 minute observations in a randomly chosen *Impatiens* stand in each invaded site. The number of *Impatiens* flowers of the stand was also counted.

In late August when the fruits of *R. sativus* were ripe we counted the number of aborted flowers and well developed fruits. We measured dry fruit weight as an estimation of plant fitness because fruit weight was highly correlated with seed set ($r^2 = 0.80$ $p < 0.001$, $n = 100$). Fruit set was calculated as the proportion of flowers that set fruit. The reproductive effort was calculated as the ratio between dry fruit weight and total aboveground biomass. Because some plants got damaged or partially eaten in the field, at the end of the experiment we only had complete data for 10 sites.

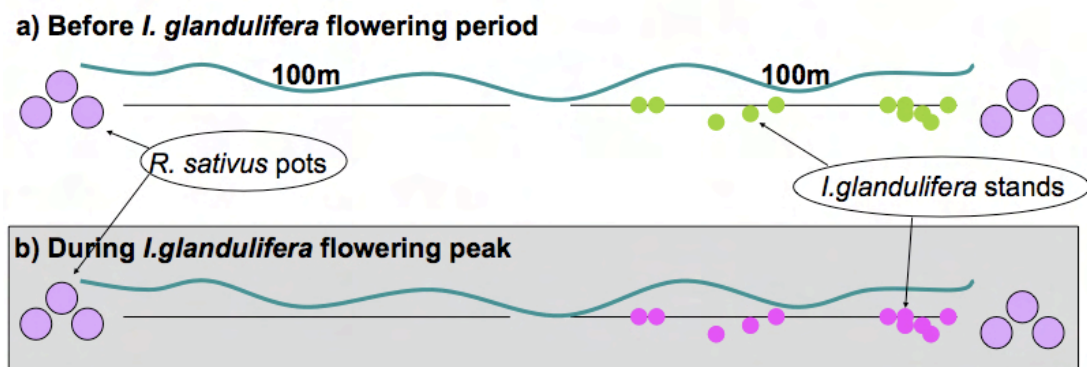


Figure 5.2: Along each riverside (blue line) we placed two permanent 100 m long transects at a distance of 100 m., one invaded by *Impatiens glandulifera* and the other not. We surveyed the two transects before *I. glandulifera* flowering (a; green small circles) and during its flowering peak (b; pink small circles). The three big purple circles indicate the proportion of the three experimental *Raphanus sativus* pots.



Statistical analysis

Statistical analyses were conducted with STATISTICA (StatSoft 2001) unless another program is specified. Data were log arcsin transformed when necessary to meet the assumptions of parametric statistical analysis.

Effects of invasion and phenology

For each plot in the pollinator surveys we calculated pollinator richness (number of species), plant richness and total number of visits and interactions. The effect of invasion before and during *Impatiens* flowering on these dependent variables was compared by repeated measures ANOVA with invasion as a fixed factor and site as a random factor. Number of visits was also decomposed in number of visits of the following pollinator guilds: honeybees, bumblebees, wild bees and syrphids.

For the *R. sativus* experiment, total number of pollinator visits, visits of each pollinator guild, fruit set and reproductive effort were analysed as dependent variables with the same ANOVA type model.

To test whether *Impatiens* plants received a number of visits comparable to average visitation rates on native species, we compared the visitation frequency received by the invader with a normal distribution fitted to the number of visits received to native species for each site.



Combined effects of invasion and landscape context

Simple regressions were used to assess the correlation between richness and abundance (i.e. number of visits) of pollinators. For bumblebees and honeybees we used the proportion of natural land cover at 3000 m radii and for wild bees and syrphids at 500 m radii based on earlier studies indicating that these scales were most relevant for the respective pollinator guilds (Steffan-Dewenter et al. 2002, Westphal et al. 2006).

To further explore whether differences due to invasion within the landscape context differed in the pollinator assemblage for the community survey and for the *R. sativus* experiment, we performed a Mantel test for the data before and during *Impatiens* flowering. This test compares similarities of different variables among all plots. We compared the following three similarity variables among plots: 1) plant-pollinator interaction composition 2) landscape and 3) invasion.

The similarity matrix of the plant-pollinator interaction composition (i.e. identities and frequencies of interactions) between all pairs of plots was estimated with the Kulczynski's distance coefficient ($K = 1/2(W/A + W/B)$; where W is the sum of the minimum visits of the various species, this minimum being defined as the visits at the site where the species is the rarest and, A and B are the sums of the visits of all species at each site, respectively). The index was calculated between all pairs of plots. This index is semimetric, meaning that it accounted for differences in the contribution to the coefficient between abundant species and rare species (Legendre and Legendre 1998). We also use the resulting pair-wise similarity matrix as input for non-metric multidimensional scaling (NMDS), an ordination technique used to provide a visual representation of the pattern of proximities among plots.

The landscape similarity matrix was measured using the Proportional similarity index P_s , calculated as $P_s = 1 - 0.5 \sum |P_i - P_{ni}|$; where P_i is the frequency of each land cover in invaded plots and P_{ni} the frequency of each land cover in not invaded plots.



Finally, the invasion similarity matrix was also constructed with the Proportional similarity index of *Impatiens* cover at each plot. Statistical significance was tested by Bonferroni permutations (n=1000) with the software PASSAGE (Rosemberg 2001).

Effects on bumblebees

As bumblebees were the most abundant pollinators (almost half of total visits) and were commonly shared between native plant species and the invasive *Impatiens*, we tested whether invasion, landscape context and total flower abundance influenced bumblebee visitation rates to the whole plant community and to native species during *Impatiens* flowering.

For this purpose we used a Linear Mixed Effect Model fitted by REML in R (R Development Core Team 2008). We present here the minimum adequate model with the low AIC (Akaike's Information Criterion; Akaike 1974) that is: N° Visits= percentage of natural habitats in a 3000 m radius + total flowers in transect + *Impatiens* cover + error (site). To test whether invasion and landscape context affected the visitation rates while accounting for site clustering, we considered site as a random block factor and the other variables as fixed factors.

Results

Effects of invasion and phenology

During the pollinator survey, the number of flowering plant species was larger before than during *Impatiens* flowering (Table 5.1 and 5.2). For example, species from the genera *Ranunculus*, *Rubus*, *Lotus* and *Stellaria* had an early flowering. For others, only its late flowers from overlapped with the flowering peak of *Impatiens* (i.e. *Stachis palustris*, *Vicia* spp and some Apiaceae and



Asteraceae). Finally, just a few plant species had a flowering peak completely overlapped with the invader (*Centaurea jacea*, *Epilobium angustifolium*, *Symphytum officinale*).

During the pollination survey we found a total of five pollinator taxa visiting *Impatiens*: three bumblebee species, one wasp and one honeybee species. In contrast, overall native plants were visited by 48 pollinator taxa during *Impatiens* flowering, including honeybees, 12 wild bees, 4 bumblebees, 7 syrphids and a few beetles, flies, butterflies and wasps. However, particular native species received on average (\pm SE) 6.26 ± 1.48 pollinator taxa per plant species, a value that did not differ from that of *Impatiens* (5 taxa). No pollinators visited exclusively *Impatiens* flowers.

Table 5.1: Network parameters (mean \pm S.E.) for non-invaded and invaded plots before and during *Impatiens glandulifera* flowering.

	Non-invaded / Before	Invaded / Before	Non-invaded / During	Invaded / During
Number of plants	4.79 \pm 1.63	5.43 \pm 2.34	2.71 \pm 1.32	3.64 \pm 1.55
Number of pollinators	5.71 \pm 2.49	5.79 \pm 2.83	1.71 \pm 1.33	2.93 \pm 2.13
Number of interactions	7.71 \pm 3.65	8.71 \pm 6.50	2.21 \pm 1.89	4.36 \pm 3.03
Number of visits	13.79 \pm 8.40	13.86 \pm 12.70	5.14 \pm 6.67	30.64 \pm 19.17
Number of visits to natives	13.79 \pm 8.40	13.86 \pm 12.70	5.14 \pm 6.67	6.50 \pm 6.89

We found more pollinator species and a higher number of interactions before than during *Impatiens* flowering. Moreover, we found more pollinator species and more interactions in invaded sites than in non-invaded plots (Table 5.1 and 5.2). Similarly, there were more visits in invaded plots than in non-invaded plots. The significant interaction between invasion and the phenological timing indicated that invasion had a positive effect on visitation only during *Impatiens* flowering. Nevertheless, this increase is due to visits to invasive plants, not to the natives for which there were not significant differences (Table 5.1 and 5.2, Figure 5.3). Pollinator visits to *Impatiens* accounted for 38 % of the total visits in all sites.

Overall we recorded a total of 887 visits from which 464 were from bumblebees. When looking to each specific pollinator guild, we found more visits in invaded plots than in non-invaded ones for bumblebees and honeybees and a significant interaction between invasion and the phenological



timing indicating that invasion had a positive effect on its visitation only during *Impatiens* flowering. However for wild bees and syrphids we found more visits before than during *Impatiens* flowering, and no invasion effect (Table 5.2).

Impatiens received on average more visits (mean \pm SE, 25.7 ± 4.7) than any particular native species (before *Impatiens* flowering: 10.72 ± 7.2 and 9.7 ± 5.2 visits in non-invaded and invaded plots, respectively and during *Impatiens* flowering: 6.0 ± 1.5 and 7.64 ± 2.1 visits in non-invaded and invaded plots, respectively; z tests: $p < 0.001$ in all sites).

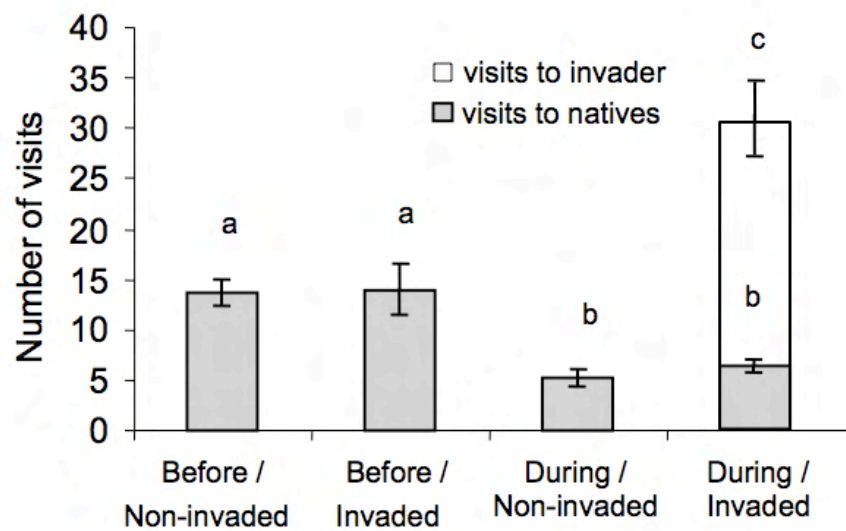


Figure 5.3: Number of visits (\pm SE) in non-invaded and invaded plots before and during *Impatiens glandulifera* flowering. The visits in invaded plots during flowering are decomposed in visits to the native and visits to the invader.

Effect of invasion in a landscape context

We found no correlation between pollinator richness and percentage of natural areas ($p > 0.1$). However, we found a negative correlation between the number of visits of bumblebees and honeybees recorded and the percentage of natural land cover in 3000 m radius before the *Impatiens* flowering peak ($r_2 = 0.25$, $p = 0.003$), but this correlation disappeared during invasion



($p = 0.5$, Figure 5.4). In contrast, we did not find any correlation for wild bees or syrphids in 500 m radii neither before nor during *Impatiens* flowering ($p > 0.1$).

When comparing interaction composition, landscape similarity and invasion effect using a Mantel test, we found that plots with similar land cover showed a similar interaction composition (i.e. identity and frequency of visits) before flowering (Mantel correlation= 0.18; $p = 0.02$). However, during *Impatiens* flowering this correlation disappeared (Mantel correlation= 0.02 $p = 0.2$) and interaction composition and invasion effect were highly correlated (Mantel correlation=0.23 $p = 0.001$). The NMDS shows an aggregation of the invaded plots during *Impatiens* flowering indicating a similar interaction composition of invaded sites (Figure 5.5).

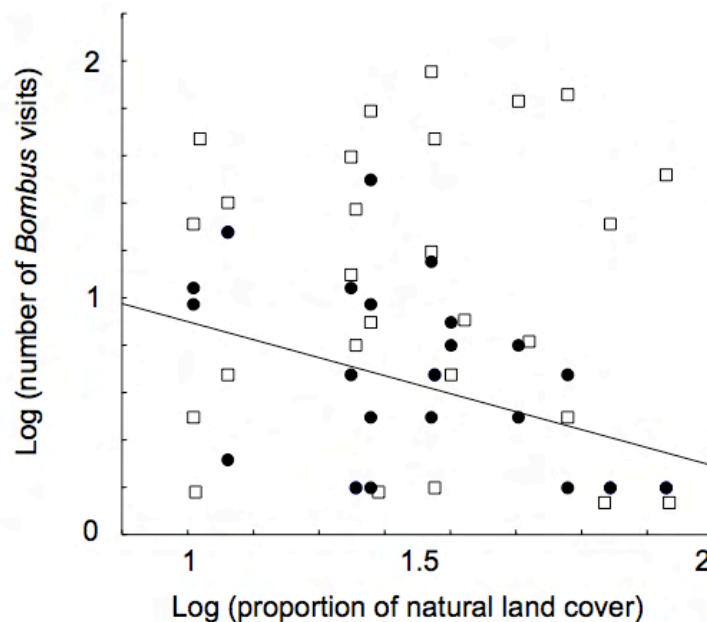


Figure 5.4: Linear regression between the number of bumblebee visits before (black dots and black line) and during (white squares) *Impatiens glandulifera* flowering and proportion of natural land-cover in a 3000 m neighbour area.



Effects on bumblebees

The number of bumblebee visits before *Impatiens* flowering was negatively correlated with the percentage of natural land cover at a 3000 m radii ($t = 2.36$, $df = 12$, $p = 0.035$; see simple regression in Figure 5.4) and positively correlated with the total amount of flower units in the transect ($t = 6.38$, $df = 12$, $p > 0.0001$). However, it was not correlated with *Impatiens* cover ($t = 1.03$, $df = 12$, $p = 0.3$). In this model, the site factor explained 28% of the variance. In contrast, during *Impatiens* flowering, the relationship between bumblebee visits and percentage of natural land cover disappeared. The percentage of natural areas ($t = 1.07$, $df = 12$, $p = 0.3$; see simple regression in Figure 5.4) and the total number of flowers ($t = 0.36$, $df = 12$, $p = 0.7$) were not correlated with bumblebee visits, but we found a significant positive correlation with *Impatiens* cover ($t = 4.07$, $df = 12$, $p = 0.0015$). In this model, the site factor explained 86% of the variance.

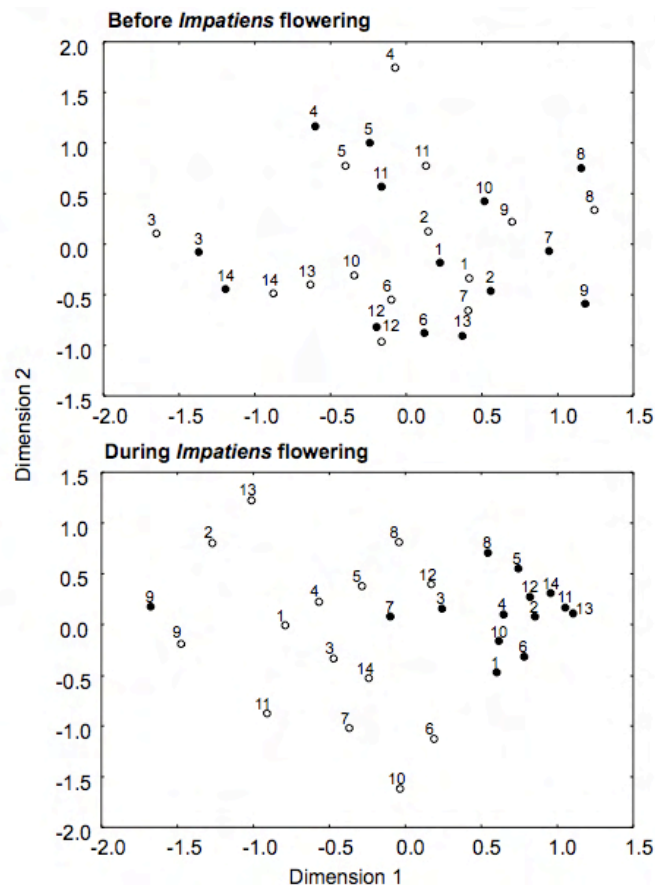


Figure 5.5: Result of the Multidimensional Scaling (NMS) for the community survey. The invaded sites (black dots) and non-invaded sites (white dots) did not show any group pattern before *Impatiens glandulifera* flowering, but grouped during *I. glandulifera* flowering peak. Each number represents a sampling paired site.





Table 5.2: Repeated measures ANOVA for the principal plant-pollination interactions parameters of the communities, showing the time effect (before and during *Impatiens glandulifera* flowering), the invasion effect (invaded and non-invaded) and its interaction. Site was included as a random factor. Significance: (ns) when $p > 0.005$, (*) when $0.01 < p < 0.05$, (**) when $0.001 < p < 0.01$ and (***) when $p < 0.001$.

	df	N° plant species	N° pollinator species	N° of interactions	N° visits	N° visits from bumblebees	N° visits from honeybees	N° visits from wild bees	N° visits from syrphids	N° visits to natives
Time	1	F=31.59 ***	F=67.3 ***	F=43.42 ***	F=1.23 ns	F=10.34 ***	F=6.02 *	F=15.31 *	F=23.92 ***	F=10.14 **
Invasion	1	F=4.20 ns	F=4.78 *	F=6.92 *	F=32.4 ***	F=35.46 ***	F=2.1 ns	F=0.03 ns	F=0.11 ns	F=1.38 ns
Time x invasion	1	F=2.05 ns	F=7.03 **	F=7.96 **	F=67.15 ***	F=31.34 ***	F=4.62 *	F=0.64 ns	F=0.25 ns	F=0.14 ns
Site	13	F=3.11 *	F=5.10 **	F=3.23 *	F=1.96 ns	F=1.53 ns	F=7.2 ns	F=2.3 ns	F=1.75 ns	F=1.43 ns

Table 5.3: Repeated measures ANOVA for the principal pollination interactions parameters in *Raphanus sativus* experiment, showing the time effect (before and during *Impatiens glandulifera* flowering), the invasion effect (invaded and non-invaded) and its interaction. Site was included as a random factor. Significance: (ns) when $p > 0.005$, (*) when $0.01 < p < 0.05$, (**) when $0.001 < p < 0.01$ and (***) when $p < 0.001$.

	df	N° visits	N° Visits from bumblebees	N° Visits from honeybees	N° Visits from wild bees	N° Visits from syrphids	df	Fruit set	Reproductive effort
Time	1	F=0.99 ns	F=3.36 ns	F=0.95 ns	F=3.82 ns	F=0.37 ns	1	F=0.13 ns	F=1.60 ns
Invasion	1	F=1.24 ns	F=1.51 ns	F=0.63 ns	F=0.71 ns	F=0.89 ns	1	F=0.01 ns	F=0.49 ns
Time x invasion	1	F=0.01 ns	F=1.52 ns	F=0.01 ns	F=0.48 ns	F=1.82 ns	1	F=0.02 ns	F=0.05 ns
Site	13	F=1.04 ns	F=1.87 ns	F=1.05 ns	F=0.85 ns	F=0.74 ns	9	F=3.9 ns	F=2.22 ns

***Raphanus sativus* experiment**

We recorded a total of 636 visits to *R. sativus* before *Impatiens* flowering most of which were from syrphids (52%), During *Impatiens* flowering we recorded a similar amount, 533 visits, also mostly by syrphids (58%). There were no significant differences in the total number of visits to *R. sativus* between invaded and non-invaded plots and before or during *Impatiens* flowering (Figure 5.6, Table 3). There were neither significant differences on visitation rates from different pollinator guilds (Table 5.3).

Fruit set was positively correlated with the number of observed visits both before *Impatiens* flowering ($r_2 = 0.58$ $p < 0.05$) and during flowering ($r_2 = 0.47$ $p < 0.05$). However, fruit set, neither reproductive effort differed significantly between invaded and non-invaded plots before and during *Impatiens* flowering (Figure 5.6, Table 5.3).

During the 15 minute observations, we recorded a total of 1605 visits to *Impatiens* compared to 636 visits to *R. sativus*. These visits were mainly from four bumblebee taxa (82%) and honeybees (16%). The remaining 2% of the visits were made by wasps and we recorded a single wild bee (*Sphcodes* sp.) visit. The number of visits to *Impatiens* was positively correlated with its cover ($r_2 = 0.54$; $p < 0.05$) but not with the percentage of natural land cover in 3000 m radii ($r_2 = 0.13$; $p > 0.1$).

The Mantel test showed a correlation between number of visits to *R. sativus* and landscape context before *Impatiens* flowering (Mantel correlation = 0.26, $p = 0.003$) but not during flowering (Mantel correlation = 0.08, $p = 0.2$). The analysis reinforced that invasion did not affect visitation rates on *R. sativus* (Mantel correlation = 0.058, $p = 0.6$). The NMDS did not show any aggregation (Figure 5.7).



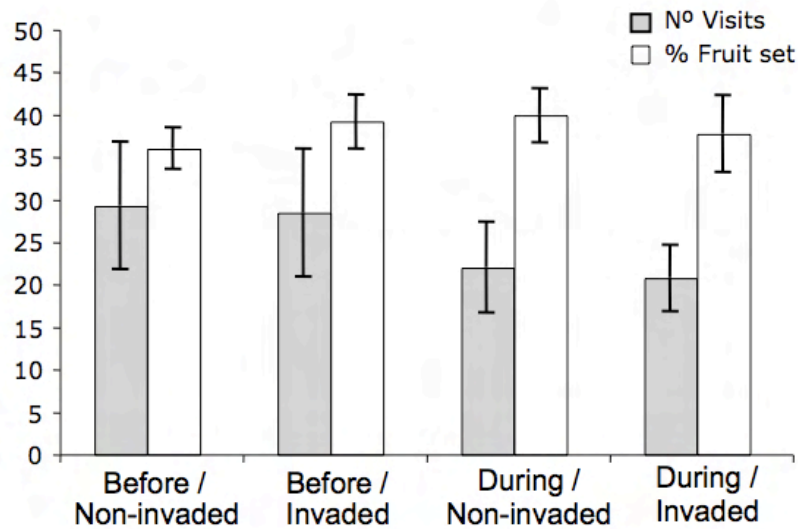


Figure 5.6: Number of pollinator visits and percentage of fruit set (\pm SE) of *Raphanus sativus* in non-invaded and invaded plots before and during *Impatiens glandulifera* flowering.

Discussion

Contrary to our expectations, the effects of the local invasion of *Impatiens* and landscape context on flower visitation of native plants were independent, predominating the mass-flowering effect of the invader. Moreover, there were no competition neither facilitation for pollinators between the native plants and the invader. Here, we discuss the combined implications for the different pollinators guilds and for native plants.

Implication for pollinators

The landscape of all our plots was very similar in mean patch area and perimeter of the ecotones and the principal differences were the proportion of natural/agricultural habitats. Agricultural fields provide mass-resources for mobile pollinators as bumblebees (Westphal et al. 2003), while natural habitats constitute a high diversity of flowering plants and important nesting sites for wild bees (Steffan-Dewenter et al. 2002). As shown by the Mantel test, plots with similar land cover



also had a similar pollinator composition before *Impatiens* flowering, indicating an important effect of the landscape on local pollinator communities.

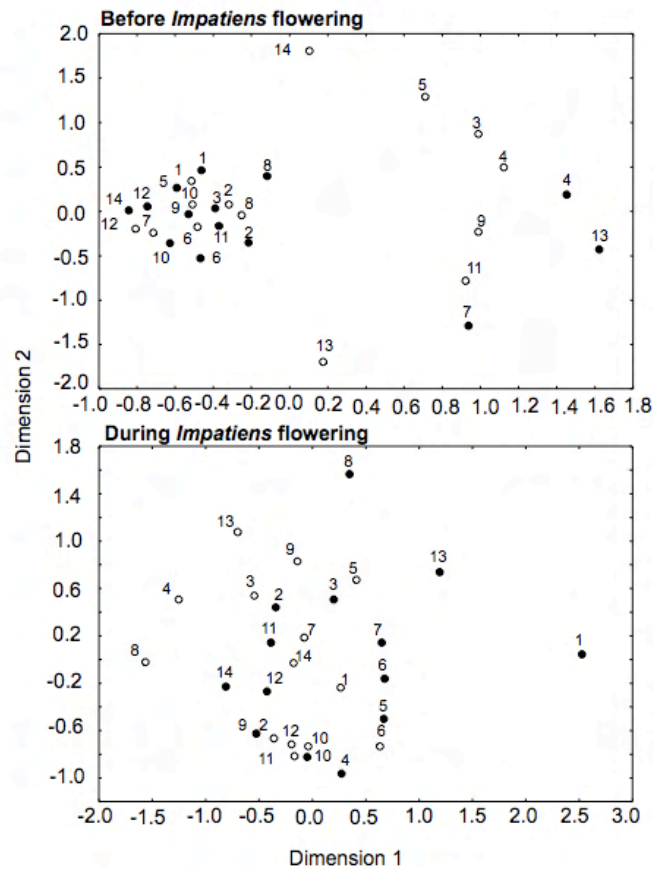


Figure 5.7: Result of the Multidimensional Scaling (NMSD) for the *Raphanus sativus* visits. The invaded sites (black dots) and the non-invaded sites (white dots) did not show any group pattern both before and during *Impatiens glandulifera* flowering. Each number represents a sampling paired site.

Concretely, we found enhanced bumblebee and honeybee densities in landscapes with higher proportions of agricultural land, but in contrast to previous studies (Steffan-Dewenter et al. 2002), we did not find any correlation between syrphids and wild bees, and the landscape context. That could be due to the fact that all our plots were located in riparian habitats and not in agricultural field margins as in previous studies. Therefore, for these two guilds, the regional landscape context is not as important as the immediate habitat.

Interestingly, the correlation between pollinator densities and landscape composition disappeared during the *Impatiens* flowering peak. At that time, pollinator composition was dependent on the



invasive cover as reflected by the Mantel test (see the NMDS, where non-invaded sites were clustered). When focusing on bumblebees, the positive relationship between agricultural land cover disappeared when *Impatiens* was flowering. All invaded plots attracted a large number of bumblebees independently of the landscape context where they were located. Thus, *Impatiens*, by its potential to attract bumblebees, make less severe landscape-related differences in bumblebee abundances late in the season. Recent studies have indicated that bumblebees benefit from mass-flowering crops in agricultural landscapes (Westphal et al. 2003, Herrmann et al. 2007). *Impatiens* mass-flowering could have a similar local effect than mass-crop flowering enhancing bumblebee populations i.e. by increasing the number and size of hibernating queens in agriculturally dominated landscapes. However, the effect of the invader on bumblebee population dynamics remains to be investigated.

Implications for native plants

Impatiens increased the visitation rate to the community of highly mobile pollinators like bumblebees and honeybees. Due to its flower morphology *Impatiens* is only accessible for rather large bees with intermediate or long tongues such as *Bombus*, *Apis* and *Vespa*. Nonetheless, *Impatiens* is well integrated in the plant-pollinator network, because even if it was visited by few pollinator taxa, visitations took place at high frequencies. However, it does not act as a magnet species as found in other regions (Lopezaraiza-Mikel 2007) because its presence did not change total number of visits to native co-flowering plants. Therefore, despite that *Impatiens* received on average much more visits than native species, the observational survey do not support the hypothesis of competition for pollinators at any landscape context.

Raphanus sativus received efficient visits from various pollinator guilds (Conner et al. 1995) but evidence for pollination limitation has also been reported (Steffan-Dewenter and Tschardt 1999, Albrecht et al. 2007). Butterflies and bees are the most efficient pollinators on *R. sativus* and one visit per flower could be enough to assure 40% of seed set (Conner et al. 1995). In our study, co-flowering *Impatiens* stands did not reduce or increase visitation rates or seed set of *R. sativus*



despite that more pollinators were attracted to the community. Therefore, the experimental setting also contradicts competition for pollinators. Effective competition might only take place with certain specialist plant species with completely overlapping flowering phenology and pollinator syndromes (Chittka and Schürkens 2001). To identify such invasion-sensitive native plant species is a crucial task to improve conservation strategies against invaders.

A previous study found that *Impatiens* compete with experimentally added potted *Stachys palustris* plants (Chittka and Schürkens 2001), but we found that in natural communities the flowering peaks of these two species did not overlap. Our combined observational and experimental approaches suggest that some experimental settings are not realistic, even if they demonstrate that competition for pollination can occur experimentally. In fact, as for most invasive plants (Celesti-Grapow 2003, Lloret et al 2005), the flowering phenology of *Impatiens* was different from the invaded plant community. In our plots, the number of flowering plants during *Impatiens* flowering peak was lower than before the flowering peak indicating that *Impatiens* fills a late-seasonal gap in flowering phenology with potential beneficial effects particularly for social bees. Although the number of total visits to native plants were lower during the invasion flowering peak than before flowering, experimental *R. sativus* pots received the same amount of visits before as during *Impatiens* flowering. This experimental approach, allowed to discriminate for phenological effects on native species, and indicates that the decrease in visitation rates to native plants during *Impatiens* flowering was more dependent on phenological shifts, than on the invasion effect.

Conclusions

As predicted, different native pollinators guilds responded differently to the landscape context. Bumblebee densities were higher in riparian habitats surrounded by agriculturally dominated landscapes and in invaded plots, in contrast the number of wild bees and syrphids was independent from the landscape context and local plant invasion. Interestingly, the high abundance of bumblebees on invaded plots masked off landscape effects, being the effect of *Impatiens*



independent from the landscape context. Future research should endeavor in the effects of the invader on bumblebee populations. Both the observational survey and the experimental setting suggest that *Impatiens* did not outcompete native plants for pollinators indicating that at least generalist and co-flowering species are quite tolerant to competition for pollination.



Discusión general

Resumen

Los polinizadores nativos, sobre todo aquellos con un espectro de visitas generalistas, proporcionan una vía de integración para las plantas invasoras. Tanto *Carpobrotus* como *Opuntia* dependen de los polinizadores para la reproducción sexual en el área de introducción, aunque *Carpobrotus* se vea levemente limitado por el polen. La competencia por los polinizadores con las plantas nativas depende de la especie estudiada, así, encontramos competencia con *Opuntia*, facilitación con *Carpobrotus* y no encontramos un efecto en *Impatiens*. En el caso de existir facilitación por las visitas, hemos visto que el polen invasor tiene muy pocas posibilidades de interferir en la reproducción de las plantas nativas al depositarse en muy baja frecuencia en los estigmas de las especies nativas. Finalmente, cuando evaluamos la importancia del paisaje, los efectos producidos por la invasión resultan ser independientes de los efectos del paisaje.

A la luz de los resultados obtenidos, se discutirá sobre la integración de las plantas invasoras en las redes de polinización, sus impactos sobre polinizadores y plantas nativas y sobre la estructura de la comunidad nativa, así como la importancia del contexto en estos procesos.

Integración en la nueva comunidad

Las plantas invasoras estudiadas tienen flores muy diferentes al resto de la comunidad nativa (Apéndice 1). Las dos plantas invasoras de ecosistemas Mediterráneos, *Carpobrotus* y *Opuntia*, tienen flores grandes, con forma de bol, que se ajustan al síndrome clásico de abeja supergeneralista (Proctor y otros 1996). La mayoría de los polinizadores reconocen este estímulo visual (Michener 2000) y son atraídos a él (Kevan y Baker 1983). Además, en el caso de *Carpobrotus* las flores reflejan un patrón de la radiación UV (Carrasco) que atrae a las abejas (Menzel y Shmida 1993, Chittka 1996), al igual que muchas otras flores del Mediterráneo (Menzel y otros 1997). En cambio, las flores de *Impatiens* tienen una corola mucho más restrictiva que las



otras dos plantas invasoras estudiadas, que criba las visitas de ciertos polinizadores (Proctor y otros 1996, Valentine 1978). A pesar de que tienen gran cantidad de polen, ni *Carpobrotus* ni *Opuntia* presentan importantes cantidades de néctar, sin embargo *Impatiens* sí presenta néctar en abundancia y es muy rico en azúcares (Chittka y Shurkens 2001). Pese a estas diferencias entre las flores de las plantas invasoras, las tres plantas reciben muchas más visitas que las plantas nativas. Las plantas nativas coexistentes en todas las comunidades invadidas tienen flores más pequeñas que las invasoras, aunque encontramos tanto especies con una morfología floral similar (Cistaceae) como especies con flores muy restrictivas (Labiatae), la mayoría de los cuales tienen importantes cantidades de néctar (Bosch y otros 1997, Petanidou y otros 2006). Por tanto, la principal diferencia que presentan las tres plantas estudiadas respecto a la comunidad receptora es el tamaño de sus flores. Además, dos de ellas, *Opuntia* e *Impatiens*, poseen una fenología floral tardía, cuando la floración de las plantas nativas empieza a declinar.

A pesar de esta diferencia en la morfología y fenología floral, las flores de las especies invasoras son visitadas por los mismos polinizadores que las plantas nativas, integrándose en la comunidad receptora (Memmot y Waser 2002). *Carpobrotus* comparte todos sus polinizadores con las plantas nativas, y es visitado por más de un 40% de los taxones de polinizadores. *Opuntia* también es visitada por los mismos polinizadores que las plantas nativas (más del 30% de taxones), a excepción de una abeja carpintera (*Xilocopa violacea*), que no visitó ninguna otra especie nativa. *Impatiens* en cambio, que tiene una corola más restrictiva, sólo es visitada por 3 géneros, pero estos son también los polinizadores principales de la comunidad nativa.

No hay mucha información sobre qué polinizadores visitan *Carpobrotus* en su área de distribución nativa, pero los principales grupos incluyen abejas solitarias, abejas de la miel, abejas carpinteras y numerosas especies de escarabajos (Malan y Notten 2006). En California *C. edulis* es visitado principalmente por escarabajos (Vilà y D' Antonio 1998), y en Francia, Suehs y otros (2005) citan algunos taxones, similares a los que hemos encontrado visitando *Carpobrotus* en nuestra área de estudio tales como *Bombus terrestris*, *Halictus* spp. y *Anthidium* spp. Aunque *Carpobrotus* reciba muchas visitas y sus principales polinizadores carguen de manera eficiente su polen la producción de semillas de *Carpobrotus* está ligeramente limitada por el polen. Gran parte de estas visitas son



de escarabajos, que posiblemente contribuyen más a la autopolinización que a la polinización cruzada, debido al largo tiempo que invierten en cada flor. Además, la naturaleza de híbrido entre *C. edulis* y *C. acinaciformis* en que se encuentra hace que probablemente presente un cuajado de semillas bajo puesto que se han detectado altas proporciones de polen no viable. En la zona encontramos tanto abejas carpinteras como abejas de la miel. Las primeras no visitan *Carpobrotus* a pesar de visitarlo especies próximas en su área de distribución nativa, y las segundas, pese a ser propensas a interactuar con las plantas generalistas formando complejos supergeneralistas (Hanley y Goulson 2003, Gzhul 2002, pero ver Olesen y otros 2002), realizan muy pocas visitas a *Carpobrotus*, encontrándolas principalmente visitando *Rosmarinus officinalis* y *Lavándula stoechas*. Por lo tanto, estos dos grupos de polinizadores no contribuyen como se esperaba a la visita de *Carpobrotus*.

El género *Opuntia* está claramente asociado a la polinización por abejas y se ha sugerido su coevolución con al menos dos géneros de abejas solitarias (Grant et al. 1979, Reyes-aguero 2006). En Florida, *O. stricta* es visitada principalmente por abejas solitarias (Spears 1987), pero no todas ellas realizan una adecuada polinización, siendo los insectos menores de 1.5 cm incapaces de polinizar las flores de *Opuntia* sp (Grant and Hurd 1979). En nuestras parcelas, nos encontramos con más de 15 taxones visitando *Opuntia*, incluyendo diferentes géneros de abejas solitarias, aunque principalmente reciben visitas de abejas de la miel y abejas carpinteras, que son polinizadores de tamaño grande. Las flores de *Opuntia* presentan bajas tasas de autopolinización, pero son polinizadas de manera eficiente y presentan un cuajado de semillas muy alto.

Finalmente, *Impatiens* es visitada principalmente por un género de amplia distribución como es *Bombus* sp. (Valentine 1978), que también está presente en su rango de distribución nativo. *Impatiens* es autocompatible, pero la polinización cruzada es frecuente debido al gran número de visitas que recibe (Valentine 1978).



En resumen, los polinizadores nativos proporcionan a las plantas invasoras estudiadas una vía de integración en las comunidades de introducción estableciendo interacciones eficientes con ellas y permitiendo así su reproducción sexual.

Efectos sobre la actividad de los polinizadores

Las redes de planta-polinizador han coevolucionado largo tiempo juntas, de manera que las especies están adaptadas a coexistir y han desarrollado estrategias para completar adecuadamente su ciclo vital (Thomson 2001, Jordano 1998). Estas estrategias comprenden, entre otras, la especialización de algunas interacciones, la separación fenológica entre especies, la constancia o fidelidad floral por parte de los insectos, y muchas otras que aseguran una adecuada reproducción de las plantas y recursos suficientes para los polinizadores (Proctor 1996). Sin embargo, la aparición de una nueva especie de planta con flor que no ha coevolucionado en esa comunidad puede alterar los patrones de visita de ciertos grupos de polinizadores, modificar su abundancia local, y esto repercutir en la producción de semillas de las plantas nativas.

Como hemos visto, los polinizadores incluyen fácilmente la nueva planta invasora en su dieta, y estos, además realizan una gran parte de visitas a estas plantas. Sin embargo se conoce muy poco sobre los cambios en la abundancia local de las poblaciones de polinizadores. Estudiando los patrones de visita, inicialmente podemos inferir algunos de estos cambios, sobre todo en los polinizadores más abundantes. Por ejemplo, en el caso de *Opuntia*, encontramos un incremento de visitas de abejas de la miel (*Apis mellifera*) en zonas invadidas respecto a zonas no invadidas. En las zonas invadidas por *Impatiens* también hay un gran incremento de abejorros (*Bombus* sp) respecto a las no invadidas. Estas dos especies de insectos sociales mantienen grandes colonias, y necesitan muchos recursos. Al final de la primavera y principios de verano, la oferta floral de las especies nativas y los cultivos empiezan a disminuir, pero estas dos plantas invasoras están en el pico de su floración (junio para *Opuntia* y agosto para *Impatiens*). Esto podría incrementar la supervivencia de las colonias a final de temporada, incrementar los recursos para la hibernación y por consiguiente incrementar la población de la colonia el año siguiente (Westphal y otros 2006).



Sobre las abejas solitarias es más difícil inferir efectos a partir de los censos de visitas, por su baja frecuencia de visitas. Sin embargo, el caso de las abejas carpinteras visitando *Opuntia* ilustra cómo la invasión de una planta puede mantener la presencia de un polinizador en la zona. *Xilocopa violacea* visita frecuentemente *Opuntia*, sin embargo no la encontramos visitando ninguna otra planta nativa. Aunque no disponemos de datos, *Opuntia* puede haber favorecido una ampliación de la dieta en esta abeja carpintera e incluso una expansión local de forma paralela a la expansión de *Opuntia* en los últimos 300 años. Estudios más detallados sobre los efectos en diferentes grupos de polinizadores son necesarios.

Efectos sobre las plantas nativas

Las plantas nativas que dependen de los polinizadores para reproducirse pueden verse afectadas por la presencia de una nueva especie entomófila en la comunidad. En algunos estudios concretos en los que se analiza una planta nativa focal y otra invasora, se han encontrado procesos de competencia (Chittka y Shurkens 2001, Brown y otros 2001). Sin embargo, si miramos la tendencia general a nivel de comunidad, se ha encontrado que comunidades invadidas reciben más visitas que las no invadidas (Lopezariza-Mikel y otros 2007). Cuando nos centramos solo en las visitas recibidas por las plantas nativas, en nuestros estudios encontramos casos de competencia (*Opuntia*), facilitación (*Carpobrotus*) y casos de efectos neutros (*Impatiens*) (Figura 6.1). El número de visitas está fuertemente relacionado con el cuajado de semillas (Vázquez y otros 2005), y así lo hemos encontrado en los potes experimentales plantados con *Raphanus sativus*, donde las plantas que recibían más visitas presentaban más cuajado de semillas independientemente de qué polinizadores lo visitaran. Aunque cada especie puede tener sus particularidades, a nivel de comunidad es un dato muy fiable para comparar tendencias (Shali y Conner 2006).

Un aspecto que se ha puesto de manifiesto cuando existe facilitación por el polinizador, es la posible interferencia del polen invasor con el nativo. Es decir que la facilitación por polinizadores puede acarrear competencia por el polen. En Mallorca, Moragues y Traveset (2005) no



encontraron casi deposición de polen de *Carpobrotus* en estigmas de plantas nativas. Cuando añadieron polen invasor experimentalmente, tampoco detectaron un efecto negativo en el cuajado de semillas. Nosotros, además, contrastamos que aunque la red de transporte de polen sí presenta altas frecuencias de polen invasor, éste no se deposita en los estigmas nativos posiblemente porque existe una elevada fidelidad individual entre los polinizadores y las plantas que visitan en cada vuelo (Proctor 1996). Por lo tanto, hay que tomar con precaución las inferencias sobre las consecuencias últimas de la saturación de polen en los cuerpos de los polinizadores (Lopezaraiza-Mikel y otros 2007).

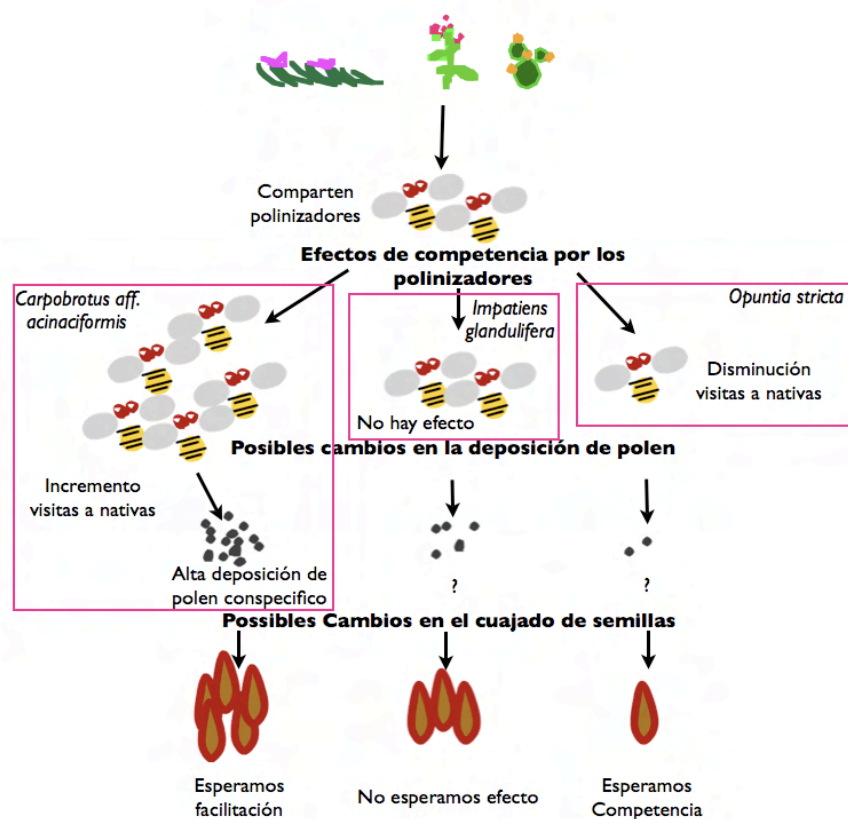


Figura 1.3: Marco conceptual simplificado de los mecanismos encontrados en las tres plantas estudiadas.

Aunque no encontremos procesos de competencia a nivel de comunidad, es importante hacer hincapié en que ciertas especies de plantas sí pueden verse afectadas negativamente. Moragues y Traveset (2005) reportan un caso de competencia entre *Carpobrotus* y *Lotus cytisoides* en Mallorca y Cittcka y Schürkens (2001) encuentran que *Impatiens* compite con *Istachis palustris* en condiciones experimentales pero no en nuestras comunidades naturales. Encontrar qué plantas



nativas pueden estar amenazadas por sus condiciones de rareza o especificidad, es clave para la conservación de la biodiversidad frente a las invasiones biológicas.

Efecto sobre la estructura la red planta-polinizador

Las comunidades estudiadas sólo presentan una planta invasora, y el estado de invasión no supera el 30% en cobertura vegetal. Esto implica que estamos estudiando procesos de invasión tempranos. Un artículo reciente de Aizen y otros (2008) encuentra que existe una correlación positiva entre los cambios en la estructura de la red de plantas y polinizadores y la abundancia de plantas y polinizadores exóticos, los cuales monopolizan la red de interacciones. El hecho de que nuestros estudios tengan un bajo grado de invasión puede explicar por qué los efectos son leves al ser las redes muy robustas (Memmot y Waser 2002, Fortuna y Bascompte 2006). A pesar de eso, encontramos ciertos cambios en parámetros tan subyacentes como es el anidamiento (Bascompte y otros 2003) en las comunidades invadidas por *Opuntia*. Las redes planta-polinizador tienden a estar anidadas, aunque en redes pequeñas es difícil detectar este patrón (Olesen y otros 2008), aunque eso no implica que el patrón no exista. En las parcelas no invadidas por *Opuntia* no detectamos una estructura anidada, sin embargo, en las parcelas invadidas sí se manifiesta el patrón anidado, debido a que *Opuntia* añade una gran cantidad de interacciones generalistas, ayudando a encajar la matriz sin que desaparezcan las interacciones con las plantas nativas. En el caso de *Carpobrotus* no encontramos diferencias en el anidamiento entre parcelas invadidas y no invadidas. Las redes de *Impatiens* fueron demasiado pequeñas para ser susceptibles de análisis.

En resumen, en las redes invadidas, en promedio, desaparecen pocas interacciones y aparecen nuevas. Las plantas invasoras tienen la capacidad de modificar las frecuencias de visita. Pero estos cambios no quedan reflejados en el anidamiento de la red, que sólo tiene en cuenta las presencias o ausencias de interacciones y no su frecuencia. Tanto *Carpobrotus* como *Opuntia* se encuentran “atadas” fuertemente a la red, mucho más que el resto de plantas nativas, eso implica que los polinizadores dependen más de las plantas invasoras que de las nativas. Esta dependencia



de ciertos taxones por la planta invasora es extrema en áreas invadidas por *Impatiens*, donde *Bombus* spp. llega a depender en más de un 90% de *Impatiens* (datos no mostrados).

La importancia del contexto

El efecto que causan las plantas invasoras sobre los ecosistemas depende del contexto ecológico (Lonsdale 1999). Por ejemplo, se ha encontrado que ecosistemas menos ricos en polinizadores pueden limitar la expansión de ciertas plantas invasoras (Parker 1997). Por tanto, analizando el contexto de la invasión, se puede explicar en parte el diferente efecto que encontramos en las diferentes plantas invasoras.

En comunidades donde la competencia por un recurso es elevada, como puede ser el caso de comunidades mediterráneas al final de la época de floración, donde hay poca diversidad y abundancia de flores, la adición de un nuevo recurso floral como pueden ser las flores de *Opuntia* puede saturar el “mercado floral”, y canalizar gran parte de las visitas. Sin embargo no solo las propiedades del ecosistema receptor marcarán el efecto que produzcan las plantas invasoras,. También dependerá de las características y la abundancia de la propia especie. Por ejemplo, *Impatiens* también posee una floración tardía, pero interacciona con un solo grupo de polinizadores, las abejas sociales. Estos polinizadores tienen un comportamiento muy característico, ya que pueden viajar grandes distancias, y sus colonias pueden crecer mucho si hay recursos abundantes (Michener 2000). Por tanto, los otros grupos de polinizadores no se ven afectados por la invasión, reduciendo el impacto sobre las plantas nativas. Finalmente, en comunidades donde hay muchos recursos, como pueden ser las comunidades de *Carpobrotus* en pleno pico de floración primaveral, los polinizadores se ven atraídos a zonas con más recursos, como pueden ser las invadidas por *Carpobrotus*, pero siguen visitando en general toda la comunidad de plantas.



Además de que los efectos que puedan tener las especies invasoras van asociados al contexto propio de la invasión, en esta tesis hemos comprobado cómo el efecto de una especie invasora, *Impatiens*, tiene la capacidad de modificar los efectos que el paisaje tiene sobre las comunidades de polinizadores. Los usos de suelo determinan la diversidad de polinizadores presentes en el área, aunque diferentes grupos de polinizadores responden a diferentes escalas de paisaje (Steffan-Dewenter y otros 2002). Las abejas sociales, que son insectos más móviles y una sola de sus colonias puede cubrir áreas muy extensas, incrementan en grandes zonas agrícolas, mientras que las abejas solitarias necesitan pequeños parches de zonas naturales. Las plantas invasoras alteran la estructura de la vegetación, cubriendo grandes extensiones. Así, en áreas invadidas encontramos un incremento de abejas sociales independientemente del paisaje circundante.

Conclusiones

- I. Las tres plantas invasoras estudiadas en esta tesis están completamente integradas en las redes planta-polinizador de la comunidad invadida, aunque siguen diferentes estrategias a la hora de establecer interacciones con los polinizadores nativos. Mientras que *Carpobrotus* y *Opuntia* atraen a una gran diversidad de polinizadores de diferentes grupos, *Impatiens* atrae mayoritariamente un tipo de polinizador muy generalista, los abejorros (*Bombus* sp).
- II. Los polinizadores son muy importantes para la reproducción sexual de *Carpobrotus* y *Opuntia*, que son ligeramente autocompatibles. A pesar de eso, la producción de semillas de *Carpobrotus* está ligeramente limitada por el polen.
- III. Las tres plantas invasoras reciben en promedio muchas más visitas que cualquier planta nativa, sin embargo, los efectos sobre la comunidad de plantas difiere mucho según la planta estudiada. *Carpobrotus* atrae una gran cantidad de polinizadores a la comunidad, incrementando el número de visitas a las plantas nativas, sobre todo de escarabajos. *Opuntia* atrae una gran cantidad de polinizadores a sus flores, reduciendo la frecuencia de visitas a las plantas nativas. Finalmente, *Impatiens* no tiene un efecto en las tasa de visitas a las plantas nativas en la comunidad.
- IV. Asimismo, en condiciones experimentales *Impatiens* no tiene un efecto ni sobre las visitas, ni sobre el cuajado de semillas de *Raphanus sativus*.



- V. En el caso de *Carpobrotus*, donde encontramos un efecto facilitador respecto a las visitas a la plantas nativas, hemos visto que los polinizadores cargan principalmente polen de la especie de planta que están visitando y que en los estigmas de las flores nativas encontramos muy poca cantidad de polen heteroespecífico. Por tanto, el polen de *Carpobrotus* tiene pocas posibilidades de interferir en la reproducción de las plantas nativas.
- VI. La invasión por *Impatiens* es más determinante a la hora de modificar la red planta-polinizador que la matriz del paisaje.
- VII. Finalmente, las diferencias encontradas en los efectos de las tres especies estudiadas revelan que predecir el impacto de las plantas invasoras sobre las redes planta-polinizador no es fácil y que es altamente dependiente tanto de la comunidad receptora, como de la especie invasora.



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Appendix I A): Flowering species composition on *Carpobrotus affine acinaciformis* invaded sites, including mean plant cover, flower characteristics and number of visits from the main pollinator guilds^d.

Flowering plant species	Family	Cover (%)	Flower size (cm)	Flower morphology ^b	Nectar ^c	Phenology	Big wild bees	Small wild bees	Bumble bees	Honey bees	Big beetles	Small beetles	Syrphids	Others
<i>Aetheorhiza bulbosa</i>	Asteraceae	1,3	3 ^a	T	-	February-April	4	6	0	0	0	27	0	0
<i>Alyssum maritimum</i>	Cruciferae	1,75	0,5	D	N	September-July	2	2	0	0	0	2	0	0
<i>Carpobrotus aff. acinaciformis</i>	Aizoaceae	37	8-10	D	N	March-June	13	9	14	7	20	70	0	1
<i>Cistus albidus</i>	Cistaceae	5,5	3-4	D	Y	April-June	15	10	1	2	4	9	1	0
<i>Cistus monspeliensis</i>	Cistaceae	10,6	3	D	Y	April-June	17	21	0	0	14	27	8	0
<i>Cistus salvifolius</i>	Cistaceae	12,6	3-4	D	Y	April-June	17	17	0	2	12	30	3	0
<i>Convolvulus althaeoides</i>	Convolvulaceae	0,5	3-4	T	Y	March- July	0	0	0	0	0	5	0	0
<i>Dorycnium pentaphyllum</i>	Leguminosae	2	0,3-0,4	P	Y	May-August	0	4	0	0	0	2	0	0
<i>Euphorbia segetalis</i>	Euphorbeaceae	2,1	0,4-0,6	D	Y	March-July	0	0	0	0	0	0	0	1
<i>Galactites tomentosa</i>	Asteraceae	2	3,5 ^a	T	Y	April-July	2	1	2	1	1	7	0	1
<i>Helianthemum guttatum</i>	Cistaceae	0,8	0,5	D	Y	March- July	1	1	0	0	0	2	0	0
<i>Helichrysum stoechas</i>	Asteraceae	3,3	0,5 ^a	T	Y	May-September	0	0	0	0	1	13	0	0
<i>Lathyrus chymenum</i>	Leguminosae	1,25	1	P	Y	April-May	4	0	0	0	0	0	0	0
<i>Lavandula stoechas</i>	Labiatae	15,58	0,3-0,4	T	Y	May-July	52	11	0	15	1	0	4	7
<i>Rosmarinus officinalis</i>	Labiatae	15,33	1	T	Y	January-December	1	0	0	0	0	0	0	0
<i>Sonchus tenerrimus</i>	Asteraceae	3,16	4 ^a	T	N	April-July	4	3	0	14	1	0	7	2
<i>Thymelaea hirsuta</i>	Thimelaeaceae	15,1	0,2	D	Y	January-April	24	25	0	0	1	30	0	0
<i>Urospermum picroides</i>	Asteracea	2,41	4-5 ^a	T	-	April-July	4	2	1	6	1	1	8	4
<i>Vicea lutea</i>	Leguminosae	0,6	1	P	-	April-May	5	8	0	0	1	20	0	0
<i>Linum maritimum</i>	Linaceae	1	2	D	Y	May-July	1	1	0	0	0	0	0	0

^aSize of the capitulum.

^bD: Disc-bowl shaped corolla, T: restrictive, tubular corolla, P: very restrictive, papilionaceous corolla.

^cY: nectar present, N: no nectar present.

^dBig wild bees: *Anthidium*, *Anophora*, *Megachile*, *Xylocopa*; Small wild bees: *Andrena*, *Halictus*, *Lassioglossum*, *Hylaeus*; Big beetles: *Oxithirea*, *Myrabilis*; Small bees: *Psilotrix*, *Cryptocephalus*, *Oedemera*, *Mordella*.



Appendix I B): Flowering species composition on *Opuntia stricta* invaded sites, including mean plant cover, flower characteristics and number of visits from the main pollinator guilds^d.

Flowering plant species	Family	Cover(%)	Flower size	Flower morphology ^b	Nectar ^c	Phenology	Big wild bees	Small wild bees	Bumble bees	Honeybees	Big beetles	Small beetles	Syrphids	Others
<i>Allium ampeloprasum</i>	Liliaceae	0,5	0.3-0.5	T	Y	June-July	0	0	0	0	0	0	0	1
<i>Brassica fruticulosa</i>	Cruciferae	1,3	0.6-0.8	D	Y	February-July	0	1	0	0	0	0	0	0
<i>Convolvulus althaeoides</i>	Convolvulaceae	3,5	3-4	T	Y	March- July	0	4	0	0	0	15	0	0
<i>Daucus carota carota</i>	Umbelliferae	10,58	0.4-0.6	D	Y	June-July	0	27	0	1	8	103	3	4
<i>Dianthus caryophyllus</i>	Caryophyllaceae	3	0.6-0.8	T	Y	June-August	0	0	0	0	0	1	0	0
<i>Echium sabulicola</i>	Boraginaceae	2	1.5-2	T	Y	March-June	0	3	0	0	0	0	0	0
<i>Eryngium campestre</i>	Umbelliferae	2,75	0,2	D	Y	May-October	0	10	0	0	0	16	0	2
<i>Helianthemum guttatum</i>	Cistaceae	0,63	0,5	D	Y	March- July	0	0	0	0	0	0	0	1
<i>Helichrysum stoechas</i>	Asteraceae	1,25	0.5 ^a	T	Y	May-September	0	0	0	0	0	3	0	0
<i>Hypericum perforatum</i>	Guttiferae	0,66	1	D	Y	May-June	1	0	0	0	0	0	0	0
<i>Opuntia stricta</i>	Cactaceae	17,33	8-10	D	N	June-July	22	11	18	33	26	11	0	9
<i>Psoralea bituminosa</i>	Leguminosae	6	0.8-1	P	Y	May-June	3	3	0	0	5	2	0	0
<i>Ruta chalepensis</i>	Rutaceae	5,75	1-1.2	D	Y	March-August	0	10	0	0	1	7	0	4
<i>Scabiosa maritima</i>	Dipsacaceae	0,36	1-1.2	T	Y	March-September	0	4	1	11	4	0	1	5
<i>Sedum sedifforme</i>	Crassulaceae	5,75	0.8-1	D	Y	July-August	1	14	1	0	1	2	0	4
<i>Sonchus tenerrimus</i>	Asteraceae	3,25	4 ^a	T	N	April-July	0	38	1	9	4	7	4	3
<i>Urospermum picroides</i>	Asteraceae	1,22	4-5 ^a	T	-	April-July	1	10	0	0	1	1	0	0

^a Size of the capitulum.

^b D: Disc-bowl shaped corolla, T: restrictive, tubular corolla, P: very restrictive, papilionaceous corolla.

^c Y: nectar present, N: no nectar present.

^d Big wild bees: *Anthidium*, *Antophora*, *Megachile*, *Xylocopa*; Small wild bees: *Andrena*, *Halictus*, *Lassioglossum*, *Hylaeus*; Big beetles: *Oxithira*, *Myrabilis*; Small bees: *Psilotrix*, *Cryptocephalus*, *Oedemera*, *Mordella*.



Appendix I C): Flowering species composition on *Impatiens glandulifera* invaded sites, including mean plantcover, flower characteristics and number of visits from the main pollinator guilds^d.

Flowering plant species	Family	Cover(%)	Flower size	Flower morphology ^b	Nectar ^c	Phenology	Big wild bees	Small wild bees	Bumblebees	Honeybees	Big beetles	Small beetles	Syrphids	Others
<i>specie 1</i>	Umbelliferae	0,2	D	1,38	-	June-August	0	0	0	0	0	0	0	0
<i>specie 2</i>	Labiatae	0,5	T	5,72	-	June-August	0	0	9	0	0	0	0	0
<i>Symphytum officinale</i>	Boraginaceae	1,5-2	T	2,38	Y	June-September	0	0	4	0	0	0	0	0
<i>Geranium sylvaticum</i>	Geraniaceae	3,5-4	D	22,22	Y	June-August	0	0	12	0	0	0	7	1
<i>Silene sp</i>	Caryophyllace	1,5-2	T	1,38	-	July-September	0	0	1	1	0	0	4	0
<i>Geranium robertianum</i>	Geraniaceae	0,2-0,3	D	4,55	Y	May-September	0	0	0	0	0	0	0	0
<i>specie 3</i>	Cruciferae	0,5	D	1,77	-	June-August	0	0	0	0	0	0	0	0
<i>Centaurea jacea</i>	Asteraceae	4a	T	9,72	Y	July-September	0	0	2	0	0	0	0	0
<i>Hypericum perforatum</i>	Guttiferae	2	D	2,77	Y	May-August	0	0	0	0	0	0	0	0
<i>Lythrum salicaria</i>	Lythraceae	3	T	15,83	Y	June-August	0	1	4	3	0	0	0	3
<i>Convolvulus sp</i>	Convolvulace	1	T	0,77	Y	June-August	0	1	1	0	0	0	3	0
<i>Mentha longifolia</i>	Labiatae	0,3-0,4	T	1,44	Y	July-September	0	0	0	0	0	0	0	0
<i>Impatiens glandulifera</i>	Balsaminace	3,5-4	T	26,6	Y	July-September	0	0	317	19	0	0	0	3
<i>Impatiens noli-tangere</i>	Balsaminacea	3	T	0,36	Y	July-August	0	0	0	0	0	0	1	0
<i>Epilobium angustifolium</i>	Onagraceae	4-4,5	T	4,72	Y	July-September	0	0	0	0	0	0	0	0

^a Size of the capitulum.

^b D: Disc-bowl shaped corolla, T: restrictive, tubular corolla, P: very restrictive, papilionaceous corolla.

^c Y: Nectar present, N: no nectar present.

^d Big wild bees: *Anthidium*, *Antophora*, *Megachile*, *Xylocopa*; Small wild bees: *Andrena*, *Halictus*, *Lassioglossum*, *Hylaeus*; Big beetles: *Oxithrea*, *Myrabilis*; Small bees: *Psilotrix*, *Cryptocephalus*, *Oedemera*, *Mordella*.

Appendix II A): Pollinators received by *Carpobrotus affine acinaciformis* and total number of visits recorded.

Species	Family	Order	Visits
<i>Andrena sp.</i>	Andrenidae	Hymenoptera	4
<i>Anthidium sticticum</i>	Megachilidae	Hymenoptera	5
<i>Apis mellifera</i>	Apoidea	Hymenoptera	7
<i>Bombus terrestris</i>	Apoidea	Hymenoptera	14
<i>Coleoptera_sp4</i>	Dasytidae	Coleoptera	1
<i>Coleoptera_sp57</i>	Dasytidae	Coleoptera	8
<i>Coleoptera_sp7</i>	Bruchidae	Coleoptera	2
<i>Cryptocephalus sp</i>	Chrysomelidae	Coleoptera	3
<i>Eucera sp</i>	Anthophoridae	Hymenoptera	1
<i>Megachille sp</i>	Megachilidae	Hymenoptera	1
<i>Halictus sp</i>	Halictidae	Hymenoptera	1
<i>Halictus sp</i>	Halictidae	Hymenoptera	3
<i>Megachile sp</i>	Megachilidae	Hymenoptera	1
<i>Mordella sp</i>	Mordelidae	Coleoptera	1
<i>Mordellistema sp</i>	Mordelidae	Coleoptera	7
<i>Oedemera flavipes</i>	Oedemeridae	Coleoptera	3
<i>Oedemera lurida</i>	Oedemeridae	Coleoptera	21
<i>Oedemera nobilis</i>	Oedemeridae	Coleoptera	2
<i>Osmia sp</i>	Megachilidae	Hymenoptera	2
<i>Oxythyrea funesta</i>	Scarabaeidae	Coleoptera	20
<i>Plagiolepis pigmaea</i>	Formicidae	Hymenoptera	2
<i>Psilothix sp.</i>	Dasytidae	Coleoptera	22
<i>Xylocopa sp.</i>	Anthophoridae	Hymenoptera	1



Appendix II B): Pollinators received by *Opuntia stricta* and total number of visits recorded.

Species	Family	Order	Visits
<i>Andrena sp</i>	Andrenidae	Hymenoptera	1
<i>Anthidium sp</i>	Megachilidae	Hymenoptera	5
<i>Anthidium sticticum</i>	Megachilidae	Hymenoptera	2
<i>Apis mellifera</i>	Apoidea	Hymenoptera	33
<i>Bombus terrestris</i>	Apoidea	Hymenoptera	18
<i>Coleoptera_sp4</i>	Dasytidae	Coleoptera	4
<i>Camponotus aetiops</i>	Formicidae	Hymenoptera	7
<i>Halictus pirennaicus</i>	Halictidae	Hymenoptera	5
<i>Hylaeus nigrinus</i>	Colletidae	Hymenoptera	2
<i>Lasioglossum sp.</i>	Halictidae	Hymenoptera	3
<i>Mordella sp.</i>	Mordelidae	Coleoptera	2
<i>Mordellistena sp.</i>	Mordelidae	Coleoptera	3
<i>Myrabilis quadripunctata</i>	Maloidea	Coleoptera	20
<i>Oedemera flavipes</i>	Oedemeridae	Coleoptera	2
<i>Oxythyrea funesta</i>	Oedemeridae	Coleoptera	6
<i>Scolia sp.</i>	Scolidae	Hymenoptera	2
<i>Xylocopa violacea</i>	Anthophoridae	Hymenoptera	15



Appendix II C): Pollinators received by *Impatiens glandulifera* and total number of visits recorded.

Species	Family	Order	Visits
<i>Vespula vulgaris</i>	Vespidae	Hymenoptera	3
<i>Bombus pascuorum</i>	Apoidea	Hymenoptera	310
<i>Bombus lapidarius</i>	Apoidea	Hymenoptera	3
<i>Bombus terrestris</i>	Apoidea	Hymenoptera	4
<i>Apis mellifera</i>	Apoidea	Hymenoptera	19



