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The unseen costs of agricultural expansion across a rainforest landscape: depauperate pollinator communities and reduced yield in isolated crops.

Thesis submitted by
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2005

for the research Degree of Master of Science
in Tropical Plant Sciences
within the School of Tropical Biology and
Cooperative Research Centre for Tropical Rainforest Ecology and Management
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Abstract

Biotic pollination services are an essential component of agricultural landscapes; approximately three quarters of the world's crop species use biotic pollen vectors to initiate or enhance fruit and seed production (Roubik 1995, Nabhan & Buchmann 1997, Kenmore & Krell 1998). Biotic pollination services are in decline, necessitating maintenance of either landscape elements required by pollinating species or the development of new ways to enhance currently managed species or potential new ones (Allen-Wardell *et al* 1998, Heard 1999).

In this empirical study I have surveyed the potential pollinators and measured fruit productivity within a tropical horticultural crop (*Annona squamosa* x *A. cherimola* cultivar (cu.) 'Hillary White': custard apple) across a northern Australian tropical landscape comprised of a matrix of agricultural land and remnant rainforest patches. I tested the relationship between floral visitor species richness and abundance and two variables; distance that a custard apple orchard was located from naturally occurring rainforest and rainfall. I also tested for a relationship between fruit productivity (initiation) and the distance a custard apple crop was located from naturally occurring rainforest.

Unfortunately, due to the absence of replication of the variable 'orchard distance from naturally occurring rainforest', all conclusions must be seen as relationships between measured floral visitor and fruit production variables and orchard location in the landscape. The mechanism/s driving these patterns could be a number of factors associated with the landscape gradient such as north-south gradient, rainfall decline or

distance that the orchard was located from naturally occurring rainforest and it will take further empirical study to qualify the most important ones. For simplicity here I have retained the variable ‘distance from naturally occurring rainforest’ or ‘crop isolation’ in the thesis as it was the variable that determined the selection of sites.

I found 19 species of floral visitors (18 beetles and 1 thrip) to female custard apple flowers; 16 of which are potentially new records for custard apple pollination. Species richness and abundance declined exponentially with distance that a crop was located to naturally occurring rainforest indicating that these floral visitors rely, at least in part, on rainforest resources in the landscape. This is some of the first evidence published (see Blanche & Cunningham 2005) of an effect of crop isolation from native habitat (landscape structure) on a pollinator assemblage other than social bees.

Fruit productivity showed the same relationship; flowers were pollen limited in all orchards but there was an exponential decline in productivity with crop isolation indicating that pollen limitation was more pronounced in orchards isolated from naturally occurring rainforest. Empirical evidence for an effect of landscape structure on pollinator assemblages and resultant fruit production has also been found in tropical crop species such as *Macadamia tetraphylla* (Macadamia nut), in southeastern Australia and *Coffea arabica* (Coffee) in Central Sulawesi, Indonesia (Heard & Exley 1994, Klein *et al* 2003).

I propose from this evidence that the configuration of landscape elements, such as remnant native rainforest, across an agricultural landscape must be considered in proposals to expand crop areas. Ignoring these elements including threshold distances between pollinator ‘sources’ and crop plants will be detrimental and costly to growers and ultimately may jeopardise the sustainability of agricultural crops.

I have also shown in this research that current hand-pollination practises essential to custard apple growers for producing a viable crop may not be ideal. Custard apple growers’ hand-pollinate female custard apple flowers using pollen sourced from either ‘Hillary White’ or ‘African Pride’ cultivar trees. I found that flowers hand-pollinated using cu. ‘African Pride’ produced larger and more symmetrical fruit (better fruit quality) than those hand-pollinated using cu. ‘Hillary White’. These parameters of a fruit are important to growers because larger and more symmetrical fruit are more valuable on the market. The difference in fruit quality was not associated with a decline in fruit quantity in flowers pollinated using cu. ‘African Pride’.

Hand-pollination is a incredibly time consuming practise and any progression in traditional hand-pollination techniques that improves fruit production either through an increase in fruit quantity or quality is likely to be embraced by custard apple growers.

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Chapter 1

Introduction

1.1 What are Ecosystem services?

Increasing global population will exert ever-increasing demands on natural systems to provide the requirements to sustain it. In conjunction with expanding population size, more and more natural systems are being altered to meet these demands. Alteration of natural systems can reduce easily measured aspects of natural systems such as the number of species and their relative abundances (species richness and diversity) (Debinski & Holt 2000, Lovei & Cartellieri 2001, Piessens *et al* 2004), but also changes intrinsic processes and conditions, such as energy transfer pathways (Aizen & Feinsinger 1994, Piessens *et al* 2004) that occur within them. These intrinsic processes and conditions underpin the tangible and non-tangible benefits that sustain human life (Daily 1997).

Tangible benefits provided by natural ecosystems in landscapes include: food, fibres, pharmaceuticals, and genetic resources, while non-tangible benefits include such things as the maintenance of soil fertility, clean water, clean air and spiritual and aesthetic values (Daily 1997, Costanza *et al* 1997). The conditions and processes that underpin most tangible and non-tangible benefits are generally known as “ecosystem functions” and include biological processes such as pollination, seed dispersal, gene flow, pest control, water purification and decomposition (Daily 1997, Costanza *et al* 1997).

Only comparatively recently have these conditions and processes been recognised as important considerations in assessing the true impact of habitat loss and habitat alteration. Indeed, the adoption of the general term "*ecosystem services*" to refer to biological processes such as pollination is an attempt to highlight the role that they play in maintaining human society. The new context in which species and ecosystems (and the ecosystem services that they provide) have been viewed, has led to an upsurge of research aimed at identifying the nature of ecosystem services, the ability of the world's ecosystems to continue to provide these services into the future and their value to the global economy.

Ecosystem services are numerous and diverse in identity and scale. Costanza *et al* (1997) identified seventeen broad groups of renewable ecosystem services (Table 1). For example, the world's forests play a vital role in regulating climate by maintaining gas composition of the atmosphere and storing and cycling water (Fearnside 2000, FAO 2001). Over half of the world's carbon dioxide is stored in forest ecosystems, with 20% stored in tropical forests alone (FAO 2001). Deforestation reduces the sink area for carbon dioxide accumulation and releases carbon into the atmosphere, which is known to contribute to the elevation of global temperatures (global warming) (Fearnside 2000, FAO 2001). Tropical forests have also been shown to be important in water cycling and maintaining rainfall regimes (FAO 2001). For example, approximately 50% of the precipitation received by the Amazon basin is from water that is evaporated within the basin itself (Salati & Vose 1984). Without the forest ecosystem, rainfall declines significantly and these effects are now being manifest in disrupted rainfall regimes observed in deforested areas of the Phillipines, North-western Costa Rica and Malaysia (Myers 1996).

Table 1. Ecosystem services and functions (taken from Costanza *et al* 1997)

ECOSYSTEM SERVICE	ECOSYSTEM FUNCTIONS	EXAMPLES
Gas regulation	Regulation of atmospheric chemical composition.	CO_2/O_2 balance, O_3 for UVB protection, and SO_x levels.
Climate regulation	Regulation of atmospheric chemical composition.	Greenhouse gas regulation, DMS production affecting cloud formation.
Disturbance regulation	Capacitance, damping and integrity of ecosystem response to environmental fluctuations.	Storm protection, flood control, drought recovery and other aspects of habitat response to environmental variability mainly controlled by vegetation structure.
Water regulation	Regulation of hydrological flows.	Provisioning of water for agricultural (such as irrigation) or industrial (such as milling) processes or transportation.
Water supply	Storage and retention of water.	Provisioning of water by watersheds, reservoirs and aquifers.
Erosion control and sediment retention	Retention of soil within an ecosystem.	Prevention of loss of soil by wind, runoff, or other removal processes, storage of silt in lakes and wetlands.
Soil formation	Soil formation processes.	Weathering of rock and the accumulation of organic material.
Nutrient cycling	Storage, internal cycling, processing and acquisition of nutrients.	Nitrogen fixation, N, P, and other elemental or nutrient cycles.
Waste treatment	Recovery of mobile nutrients and removal or breakdown of excess or xenic nutrients and compounds.	Waste treatment, pollution control, detoxification.
Pollination	Movement of floral gametes.	Provisioning of pollinators for the reproduction of plant populations.
Biological control	Trophic-dynamic regulations of populations.	Keystone predator control of prey species, reduction of herbivory by top predators.
Refugia	Habitat for resident and transient populations.	Nurseries, habitat for migratory species, regional habitats for locally harvested species, or over-wintering grounds.
Food production	That portion of gross primary production extractable as food.	Production of fish, game, crops, nuts, fruits by hunting, gathering, subsistence farming or fishing.
Raw materials	That portion of gross primary production extractable as raw materials.	The production of lumber, fuel or fodder.
Genetic resources	Sources of unique biological materials and products.	Medicine, products for material science, genes for resistance to plant pathogens and crop pests, ornamental species (pets and horticultural varieties of plants).
Recreation	Providing opportunities for recreational activities.	Eco-tourism, sport fishing, and other recreational activities.
Cultural	Providing opportunities for non-commercial uses.	Aesthetic, artistic, educational, spiritual, and/or scientific values of ecosystems.

On a smaller scale, ecosystems can provide services such as pest control to adjoining crop areas. For example, near-natural and natural forest fragments in Göttingen, Germany, support large populations of parasitoids that have the potential to significantly reduce the population of rape pollen beetle (*Meligethes aeneus*); an important pest on oil seed rape crops (Thies *et al* 2003). This pest control service is only effective as a biological control method when natural habitat areas cover at least 20% of the landscape. When landscapes are altered to the extent that natural vegetation cover falls below this threshold, chemical and imported biological control methods need to be implemented at a significant cost to the growers (Thies *et al* 2003).

1.2 Sustainability of ecosystem services

The ability of world's ecosystems to continue to provide these benefits for the world's population is uncertain. Declines in both the quantity and quality of ecosystem services and their associated benefits have been recorded globally. These declines are due to two predominant pressures from human activities (Vitousek *et al* 1997, World Resources Institute 2000): overuse of habitats through extractive practices and habitat reduction.

The first type of human pressure on ecosystems is their overuse through activities such as fishing, recreation, timber extraction and water diversion, which deplete intrinsic resources and disrupt ecosystem integrity (World Resources Institute 2000). This pressure pervades all known ecosystems on earth (Vitousek 1997). One classic example of the detrimental results of anthropogenic use of ecosystem resources is the world’s fisheries. For example, pollution, translocation and invasion of exotic species and over-fishing have depleted the fish stocks of the Black Sea to one-third of the quantity available in the 1970’s creating a less profitable and unpredictable fishing industry in the region (World Resources Institute 2000). In another case, the pink prawn (*Farfantepeneus duorarum*) fishery in the South of the Gulf of Mexico declined from approximately 25,000 tonnes a year in the mid-70’s to less than 1,000 tonnes in the year 2000 because of a combination of large scale natural disturbances and anthropogenic impacts such as overfishing of reproducers and juveniles, alteration of breeding habitats (mangrove and seagrass) and mortality associated with an oil spill (Arreguin-Sánchez 2001).

The second type of pressure on ecosystems is habitat reduction that arises as an inescapable result of the conversion of approximately 28-37% of the world’s terrestrial surface into managed ecosystems, agricultural land and human infrastructure (World Resources Institute 2000). Reduction in the area of natural ecosystems and the change in habitat connectedness (isolation) alter species composition and disrupt intrinsic processes (Aizen & Feinsinger 1994, Laurance & Bierregaard 1996, Cunningham 2000, Debinski & Holt 2000, Laurance *et al* 2002, Benitez-Malvido & Martinez-Ramos 2003, Stork *et al* 2003, Piessens *et al* 2004). One of the constant findings in fragmentation studies is the positive relationship between habitat area and species richness (Debinski & Holt 2000, Laurance *et al* 2002, Benitez-Malvido & Martinez-Ramos 2003, Piessens *et al* 2004). For example, Laurance & Bierregaard (1996) found that a tropical forest fragment contained a different and less diverse beetle community than a continuous forest site. Similarly, in Germany, Kruess and Tscharntke (2000) found that endophagous insect species richness was significantly positively related to habitat area. Changes in species composition and abundance can alter intrinsic processes provided by those species. For example, biotically pollinated plants growing in fragmented habitats receive less pollen than those species growing in larger reserves, indicating that biotic pollination services are modified in a smaller habitat area (Aizen & Feinsinger 1994, Cunningham 2000).

Reduction and modification of natural ecosystems is cause for great concern. This is because the degrees to which habitat alteration, including loss of biological diversity, will affect ecosystem functions are not yet known and are poorly understood. This makes predicting the outcomes of their loss difficult. Equally important, the precise nature of the basis of modelling these effects is unresolved. For example, we cannot be certain that ecosystem functions will change gradually (and linearly) with gradual changes associated with ecosystem modification. There is current concern that anthropogenic changes will push ecosystems past a threshold beyond which ecosystem functions can no longer be maintained (Daily 1997, Mooney & Ehrlich 1997, World Resources Institute 2000).

It is apparent that most biological systems behave non-linearly in response to environmental change. That is, interactions between components of an ecosystem are not proportional. Therefore gradual, linear change in one component does not produce a gradual linear change in another component of that ecosystem (Hilbert 2002). Hilbert, Roulet and Moore (2000) determined that for peatlands, a small change in precipitation (one that could possibly be associated with climate change) rapidly and unpredictably altered the system from an equilibrium state (no net gain or loss of carbon) to a state of either high accumulation or decay. Peat lands represent a substantial store of global carbon; 30% of terrestrial carbon in biomass over just 3% of the earth's land area (Hilbert, Roulet and Moore 2000). This non-linear interaction between peat growth and water table depth implies that small changes in precipitation could have a substantial effect on the global carbon balance.

1.3 Valuing Ecosystem Services

Extraction and use of ecosystems and reduction in their overall area are the primary mechanisms that threaten the sustainability of ecosystem services. How might the value of ecosystem services be included in decisions about these land uses?

The high degree of land conversion from intact ecosystems to other uses has occurred because the current value system places a higher emphasis on commodities that replace intact ecosystems than on the ecosystems themselves (Pearce & Moran 1994). For example, if a landowner is given a choice between conserving a patch of forest and converting that forest into agricultural production, any logical landowner should consider the rate of return from both options. The rate of return is the benefit derived from the particular activity, in this case agriculture versus conservation, including a function of the time it takes for the benefit to be received. For example a dollar received today from an activity, will represent a dollar plus a function of growth of that dollar over a period of time. The function of growth of that dollar is the interest or return accrued from investing the dollar over that period (Pearce and Moran 1994). Therefore, a dollar received today is worth more than a dollar received after a year because it has a growth function. Any agricultural endeavour will be associated with a potential economic return. However, there is no potential for economic return from a conservation option *per se* in the current market, particularly when a landowner is seeking short-term returns (Pearce and Moran 1994). Long term or large-scale benefits are often unrecognised or unvalued and so are the environmental costs associated with converting intact ecosystems into agriculture or infrastructure. Furthermore, any environmental costs that might accrue are borne by the wider community and not by the individual making a decision about land use (Hardin 1968). Thus, the landowner gains all the benefits of land conversion through profits generated on private land, but incurs only a portion of the environmental cost. Any cost-benefit analysis will reveal land-conversion as the most profitable option (Hardin 1968).

The aim of the concept of ecosystem services is to give a measure of value to native ecosystems other than their intrinsic value (Daily 1996). By doing so, a more accurate estimate of the potential losses associated with habitat modification and a more accurate valuation of ecosystems in the context of their role in providing services to economies can result (Cork and Shelton 2000).

The current value of ecosystem services to the global economy is unknown. Costanza *et al* (1997) provided a broad estimate of the value of ecosystem services based on the value of each known service per unit area of biome multiplied by the area of each biome, summed across all services and biomes. They estimated that the value of global services (Table 1) was between $16-54 trillion per annum. Costanza *et al* (1997) propose this to be the minimum potential value given that many services are unknown and the value of known services have rarely been estimated accurately, if at all. Although this estimate highlights the immense economic value of the worlds resource base, as a means of demonstrating the potential cost of ecosystem modification and loss on society, it has been criticised for being ineffectual in providing an estimate of the true cost borne by consumers because the total cost assumes that the consumer will no longer exist, that is, the cost will be the total loss of life (Dasgupta *et al* 2000). Dasgupta *et al* (2000) propose that estimating the value of incremental change in the environmental resource base is a more useful method that allows humans to assess and experience the change.

There are a number of ways that ecosystem services can be valued. One way an ecosystem service can be valued is by calculating the cost of replacement of that service if it is no longer provided by a native habitat (Goulder & Kennedy 1997). In this sense, the value of an ecosystem that provides species that consumes or parasitises agricultural pests would be estimated as the cost of implementing pest control measures to perform the same function in the absence of the ecosystem-derived species. The costs associated with replacement control measures could include chemical sprays or developing a biological control agent. A case study demonstrating the utility of this value system comes from Salzman (1998). In this case, the city of New York needed to invest capital to improve the quality of their drinking water supply. A well-vegetated catchment retains much of the nutrients and elements that are input into them and produce runoff of higher quality than a non-vegetated catchment (Brooks 1997). Water treatment plants can also perform this function. After a cost-benefit analysis for both the option to reinstate natural vegetation or construct a water treatment plant, the city decided to restore the natural watershed at a cost of $1-1.5 billion because it was a more viable alternative to installing a water treatment plant at a cost of $6-8 billion (Salzman 1998).

1.4. Ecosystem services in Australia

In Australia, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Myer Foundation have instigated six projects that aim to assess the nature and value of services provided by local ecosystems to the economies of those areas. The six projects are located at various positions across the Australian continent and are: Atherton Tablelands, north Queensland, Goulburn Broken Catchment, Victoria, Rangelands, New South Wales, Gwydir Catchment New South Wales, the Brigalow Region, Queensland and the Brisbane River Catchment, Queensland.

The project located on the Atherton Tablelands of north Queensland aims to assess the services (and disservices) provided by rainforest arthropods to agriculture. Rainforests may contain the greatest number and diversity of invertebrates per unit area than any other vegetation types; the majority of which are arthropods (Erwin 1983). Thus, this project looks specifically at the contribution of arthropods to tropical crop productivity. The services that rainforest arthropods are likely to provide are pollination and insect pest control and the principal disservice is their potential for harbouring herbivorous pests. These projects are the first attempt in Australia to promote the incorporation of the value of ecosystem services, from

Australian native ecosystems, into decisions about the management of natural resources (Cork and Shelton 2000).

The following research is a contribution to one component of the rainforest arthropod project on the Atherton Tablelands. In particular, I investigate the potential role of rainforest arthropods in custard apple (*Annona cherimola* x *A. squamosa*) crop pollination and test the effectiveness of current hand pollination practises. In light of this, the remainder of this chapter discusses: pollination as an ecosystem service, pollination in tropical horticultural crops and how pollination services can be affected by habitat alteration. Chapter 2 is background information on the study area and a review of the current understanding of custard apple flower form and function and pollination biology. These chapters are provided as general background to my empirical investigations that are structured as individual manuscripts in the remainder of the thesis.

1.5. Pollination as an ecosystem service

Pollination is the transfer of male gametes (pollen) between reproductive structures within or between plant individuals. Pollination, along with seed dispersal, represents the only mobile stage of a plants life history; these stages are therefore important and can significantly influence the reproductive success and genetic variation within plant populations (van der Pijl 1969, Williams & Adam 1994).

The mechanism for dispersing pollen between anthers and stigma varies considerably among plant species. As a general classification, two main modes of pollen dispersal describe most systems (Armstrong 1979). These are abiotic pollination and biotic pollination. Abiotic pollination is the transfer of pollen via wind, water or by gravity. Alternatively, biotic pollination is the transfer of pollen on the body of vertebrate or invertebrate animals (Proctor *et al* 1996). Biotic pollination is by far the most common and has evolved numerous times resulting in a diverse array of animal groups used by plants for transferring pollen (Proctor *et al* 1996). Records of biotic pollination agents from around the world include: birds, bats, mammals, thrips, wasps, beetles, bees, flies, moths, butterflies, cockroaches, springtails, earwigs, bugs, lacewings, dragonflies, lice, snails and slugs (Armstrong 1979, Roubik 1995, Nagamitsu & Inuoe 1997). Biotic pollination is particularly important because it is so common, and a large number of plant species rely on animal floral visitors to initiate or enhance fruit and seed production. It is estimated that biotic pollinators visit at least 90% of known angiosperms to varying degrees (Buchmann & Nahban 1996).

Approximately three quarters of the world's crop species use biotic pollen vectors (Roubik 1995, Nabhan & Buchmann 1997, Kenmore & Krell 1998). More importantly, however, at least two-thirds of commercial crop species are obligate out-crossers (Roubik 1995, Nabhan & Buchmann 1997). This means that these species will fail to produce fruit or seed in the absence of pollinators. Obligate out-crossers thereby *require* biotic pollination services to produce fruit and/or seeds.

In the context of ecosystem services, the direct contributions of pollinators to human welfare are diverse. For example, pollination services are important for initiating or enhancing commercial seed and fruit production, improving synchrony of fruit set and reducing the time to fruit maturation (Cunningham *et al* 2002). Enhanced fruit and seed production translates into higher market value for producers. This can be due to a direct increase in the quantity of goods, or an increase in price per unit for greater quality produce, or both (Cunningham *et al* 2002). For example, a well-pollinated apple, melon or tomato flower will contain more seeds and associated fruit mass, and develop a better symmetrical shape, than a poorly pollinated flower (Free 1993, Cunningham *et al* 2002). Synchronising yield and reducing the time to maturation can also benefit fruit growers because an earlier crop is more profitable on the market (Free 1993), and the period of susceptibility to pests and potential losses due to bad weather associated with long maturation times are minimised.

1.6. Pollination in tropical horticultural crops

In temperate Australia the majority of agricultural crops are of European origin. Agricultural “lore” suggests that the most broadly applicable, manageable and effective pollinator of these crops is the honeybee (*Apis mellifera*) (Cunningham *et al* 2002). However, the dominance of honeybees as pollinators in agricultural landscapes may be over-estimated. This is because there has been no previous attempt to gather census information on: the number of wild pollinator species servicing crops, their relative contribution to crop yield and the biology of the species involved (Nabhan & Buchmann 1997, Westerkamp & Gottsberger 2000, Cunningham *et al* 2002). This is especially true for services provided by native pollinators in tropical and sub-tropical horticulture. Not surprisingly, tropical horticulture contains a large proportion of fruit crops that originate from tropical regions. Examples include; durian (*Durio zibethinus*) (Peninsular Malaysia and Borneo), mango (*Mangifer indica*) (Western Ghats, India), papaya (*Carica papaya*) (north-west South America), carambola (*Averrhoa carambola*) (Indochina), custard apple (*Annona spp.*) (South America) and jackfruit (*Artocarpus heterophyllus*) (India to Malay Peninsula).

The pollination systems of tropical plant species are varied and involve a diverse array of invertebrate and vertebrate animal groups (Roubik 1995). An example of the potential number of invertebrate and vertebrate groups that may be responsible for pollination of tropical plant species is demonstrated by a community wide study of pollination systems in lowland dipterocarp forest by Momose *et al.* (1998). This survey found that social bees pollinated 32% of plant species and beetles (20%), birds (7%), butterflies and moths (4%), mammals (2%) and other diverse insects pollinated the remaining 68% (Momose et al 1998).

In the case of cultivated tropical plant species, data compiled by Roubik (1995) on the confirmed pollinators of 1330 crop species indicate that 72.7% of crop plants are pollinated by bees, but only 21.3% pollinated by honeybees (Nabhan & Buchmann 1997). In addition to bee pollination, flies (18.8%), bats (6.5%), wasps (5.2%), beetles (5.1%), birds (4.1%), moths (2.9%), butterflies (1.5%) and thrips (1.3%) all contribute to pollination in tropical agricultural crops (Nabhan & Buchmann 1997). In Australia, the biotic pollinators of many of the tropical crop species are largely unknown. The few studies that have been carried out indicate a large contribution from indigenous pollinator species (Cunningham et al 2002). For example, Heard (1993) showed that 50% of the insect visits to macadamia (*Macadamia integrifolia*) racemes in southeast

Queensland were by the native social bee *Trigona* spp. and native bee visitation was positively correlated with total fruit set per panicle. A study on the pollination of papaya (*Carica papaya*) in Australia has shown that this species is entirely dependent on pollination performed by hawk moths (family Sphingidae) for producing fruit (Morrisen et al 2003).

The custard apple (*Annona* spp.) is another example of a tropical crop that is visited by pollinator species other than bees. Custard apples are beetle pollinated and in particular are visited by beetles of the family Nitidulidae, although floral visitor records suggest native beetles of the families Mycetophagidae and Lathridiidae may also be responsible for some fruit production in northern Australia (Blanche unpublished). In southeast Queensland, custard apples are pollinated exclusively by *Carpophilus hemipterus* (an introduced Nitidulidae) (George *et al* 1989). In an experiment aimed to measure the contribution of these beetles to pollination and consequent fruit set, only 1.5% of flowers in the pollinator exclusion treatment produced fruit, compared with 5.7% fruit set in flowers where beetle visitation was unrestricted (George *et al* 1989).

The predominance of native pollinator species and invertebrates other than honeybees in pollination systems of tropical horticultural crops is significant because the managed honeybee that has been used extensively in crop production in temperate areas may not be an effective replacement in tropical crops in the event of pollinator declines or losses. Tropical crop plants often produce a specialised floral form that excludes unsuitable visitors from floral rewards, including pollen and stigmatic surfaces (Free 1993). For example, custard apple flowers form a floral chamber during female reproductive maturity with an entrance width of 1-2 mm. The chamber entrance prevents animals larger than a few millimeters in size, including honeybees, from contacting the stigmatic surface and pollinating the flower (personal observation).

1.7. Declines in pollination services.

There is a potential worldwide decline in pollination services in both agricultural and natural ecosystems (Buchmann & Nabhan 1996, Allen-Wardell *et al* 1998). The predominant reasons given for the declines are habitat loss, pesticide use and pathogens and disease (Kearns *et al* 1998, Kevan & Phillips 2001, Cunningham *et al* 2002). For example, in agricultural systems, an increase of area under cultivation causes a reduction of natural habitat (Kearns et al 1998, Kevan & Phillips 2001). Based on a simple species-area relationship, reduction in the area of natural habitat may reduce the diversity and abundance of pollinator species within that habitat (Rosenzweig 1995). Field evidence suggests that this is the case. For example, Aizen & Feinsinger (1994) demonstrated that both the frequency and taxon richness of flower visitors in fragmented native habitat declines with decreasing fragment size, while the presence of introduced pollinator species is markedly increased. This has important implications for reproduction, in both native and agricultural plant species, because in areas with declining habitat area due to agricultural crop expansion, there will be fewer pollinator species and/or individuals available to service a larger area of crop, resulting in a deficit of pollinators (Kevan & Phillips 2001). Pollinator deficits are known to limit fruit production in a number of plant species (Ayre & Whelan 1994). A deficit of pollinators leads to insufficient pollen deposition within receptive flowers and therefore results in levels of fruit and seed production that are lower than maximum potential (Ayre & Whelan 1989).

The expansion of crops also places individual plants at increasing distance from native habitat and the potential pollinators they support. The number of pollinator species or individuals servicing the crop may be limited by the dispersal abilities of the pollinators in a manner identical in operation to classic Island Biogeography theory (MacArthur & Wilson 1967, Harris 1984). If dispersal ability were limiting, a change in pollinator abundance and/or composition with increasing isolation from native habitat would also be expected. The proportion of highly dispersive species may increase and/or the abundance of pollinators may decrease with increasing crop isolation.

1.8. Pollination services provided by Wet tropics rainforests to crop production on the Atherton Tablelands

The valuable species that contribute to the productivity of fruit and vegetable crops on the Atherton Tablelands are unknown (Blanche *et al* 2002). A survey of insects in custard apple orchards near and far from rainforest suggests that there are at least five beetle species associated with tropical rainforest that may pollinate custard apple flowers (Blanche & Cunningham 2005). CSIRO based at the Atherton Tablelands, north Queensland, carried out surveys in 2001 of the potential pollinators of custard apple, macadamia nut, lychee and peanut crops growing on the Atherton Tablelands, for the pollination services component of the ecosystem services project in that region. For custard apple crops, nine orchards located at various distances (as per Chapter 2.2: *Orchard sites*) from naturally occurring rainforest habitat were surveyed for potential pollinators by collecting one hundred female flowers at nine am and inspecting them for arthropods. The data gathered in this survey indicated that eight beetle species (*Litargus* sp. (Mycetophagidae), *Lathridiid* sp. (Lathridiidae), *Carpophilous hemipterous, C. mutilatus, C. marginellus* (Nitidulidae), *Brachypeplus instriatus* (Nitidulidae) and one thrip species were the potential pollinators in custard apple orchards (Blanche unpublished). Blanche (unpublished) also determined that the composition of the potential pollinator fauna was different between orchards located within 500 metres from naturally occurring rainforest and those orchards that were isolated from rainforest habitat by between 5-24 kilometres. The differences in the potential pollinator community between these groups of orchards included both fewer individuals and species in more isolated orchards particularly fewer native species at greater distance from naturally occurring rainforest.

The results indicated that isolation of custard apple crops from rainforest habitat could result in a depauperate native pollinator community. These conclusions have important implications for orchard management because they suggest that like other crops (eg. mustard, radish, and coffee) the presence of native habitat at proximity to crops is an important factor determining the composition and abundance of a pollinator community and potentially the productivity of those crops (Steffan- Dewenter & Tscharntke 1999, Steffan-Dewenter *et al* 2002, Klein *et al* 2003).

The conclusions drawn in this particular survey, however, are based on a single custard apple flowering period.

The following empirical research was designed to evaluate the role that naturally occurring rainforest plays in providing biotic pollination services to custard apple crops on the Atherton Tablelands and also tests the effectiveness of current hand pollination practises.

1.9. Research aims

There were two main components to the research. The first component addressed how the distribution of rainforest-derived pollinators to custard apple orchards varied with respect to distance of a crop from naturally occurring rainforest habitat **(*Chapter 3*)**.

Therefore, the aim of my research was to repeat the survey carried out by CSIRO in the 2002 flowering period to establish whether the same effect of proximity to rainforest habitat would be detected in the pollinator community in a second year.

The second component of the research addresses productivity of custard apple crops. The 2001 survey of potential pollinators in custard apple orchards carried out by Blanche (CSIRO) indicated that the composition and abundance of the pollinator community was influenced by the proximity of an orchard to naturally occurring rainforest. However, whether this change in pollinator community produced a change in crop productivity was not addressed. Thus it remained unknown how crop productivity and landscape structure (configuration of various land-uses) were related, and whether isolation represented a currently unmeasured cost for land managers.

Therefore in the second component of this study I examined changes in crop productivity associated with changes in pollinator community **(*Chapter 4*)** as well as concurrently testing the effectiveness of current hand-pollination practises **(*Chapter 5*)**.

Chapter 2

Study site and study species

2.1. Atherton Tablelands

The study was carried out at nine commercial custard apple (*Annona cherimola* (Mill.) x *Annona squamosa* (L.): Family Annonaceae) orchards located on the Atherton Tablelands, north Queensland, Australia. The Atherton Tablelands is an elevated, undulating plateau that lies on the western side of steep ranges that extend along the northern Australian tropical coast between the cities of Cairns and Innisfail (Figures 2.1& 2.2). The plateau predominantly lies at elevations between 700 and 800 metres above sea level although the flanking ranges to the east contain mountains reaching as high as 1600 metres (Tracey 1982).

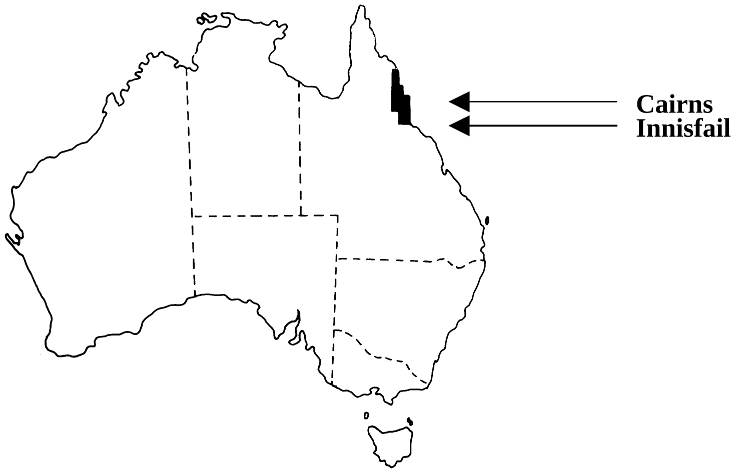

Figure 2.1. Location of humid tropical region of north Queensland, Australia (Adapted from Tracey 1982).

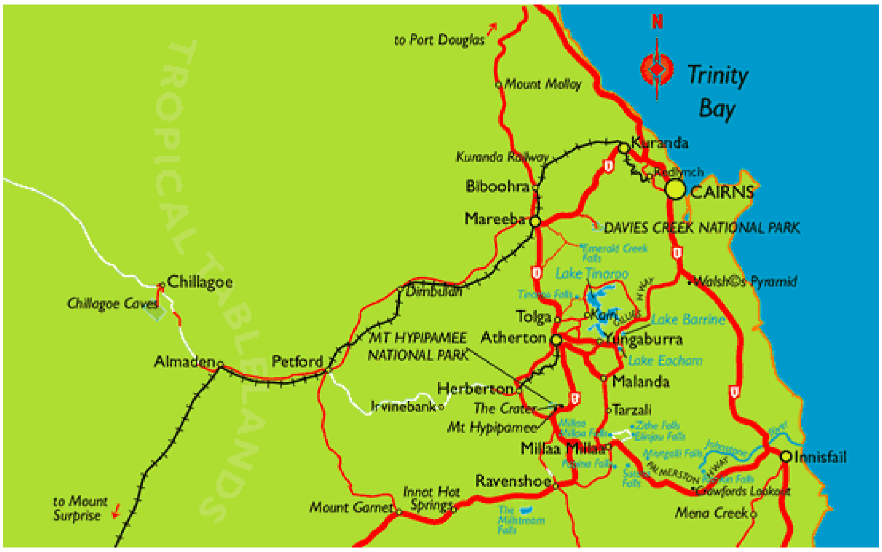

Figure 2.2. Regional map of the Atherton Tablelands

The predominant climate of the Atherton Tablelands is humid subtropical (Malcolm *et al* 1999) however climatic parameters are highly variable among regions of the Tablelands due to differences in topography. There is a distinct wet season in the humid tropical region of north Queensland because most rainfall occurs in the summer months between December and March. In contrast, the winter months between May and October are relatively dry and soil water deficits are experienced in most areas (Malcolm *et al* 1999).

The land cover of the Atherton Tablelands consists of a matrix of native vegetation, agricultural land and human infrastructure. The predominant native vegetation types are wet tropical rainforest, tall sclerophyll forest and open sclerophyll woodland. Open sclerophyll woodlands occur in areas where mean annual rainfall is less than 1400 mm (Kershaw 1974, Rainforest Conservation Society of Queensland 1986). The wet tropical rainforest types of the Atherton Tablelands are part of a larger belt of rainforest that extends between Townsville and Cooktown. This belt forms the largest continuous tract of rainforest remaining in Australia (Wet Tropics World Heritage

Area) (Tracey 1982). These rainforests are "floristically and structurally the most diverse in Australia" and contain approximately 1160 species of higher plants from 516 genera and 119 families (Rainforest Conservation Society of Queensland 1986). Tracey and Webb (1975) classified seventeen rainforest types in the Wet Tropics region. Those most common in the study area are complex notophyll vine forest and simple notophyll vine forest (Tracey 1982). The wet tropical rainforest types also contain the highest diversity of faunal species for any region in Australia, including 60% of Australia's bat species, 18% of Australia's bird species and 62% of Australia's butterfly species (Rainforest Conservation Society of Queensland 1986).

The rainforests of the Atherton Tablelands have undergone significant reductions in total area from the time of settlement in the early 1800's. Land has been cleared for agriculture, particularly sugar cane, dairy and maize and cleared for mining settlements and logging (Cassells *et al* 1986). Since settlement, land clearing has reduced the original cover of wet tropical rainforests within the Townsville to Cooktown tract to approximately 60% of the pre-settlement extent (Tracey 1982). On the Atherton Tablelands especially, this has resulted in small fragments remaining within an agricultural matrix (Tracey 1982).

The area of land under cultivation for agriculture on the Atherton Tablelands is an estimated 30 327 ha. The predominant agricultural land-use is crop production. There is a high diversity of crops produced including sugar cane, cereals, fruit, vegetables, tobacco, coffee, flowers and tea tree (Advance Cairns 2004). Crop production is the largest commodity group in the Atherton Tablelands region; the income from crop production amounted to a gross value of $123.6 million in 1999-2000 (Department of Transport and Regional Services 2002).

2.2. Orchard sites

The nine orchard sites were located along a northwest to southeast transect across the Atherton Tablelands between the township of Biboohra and the Wongabel State Forest (Figure 2.3.).

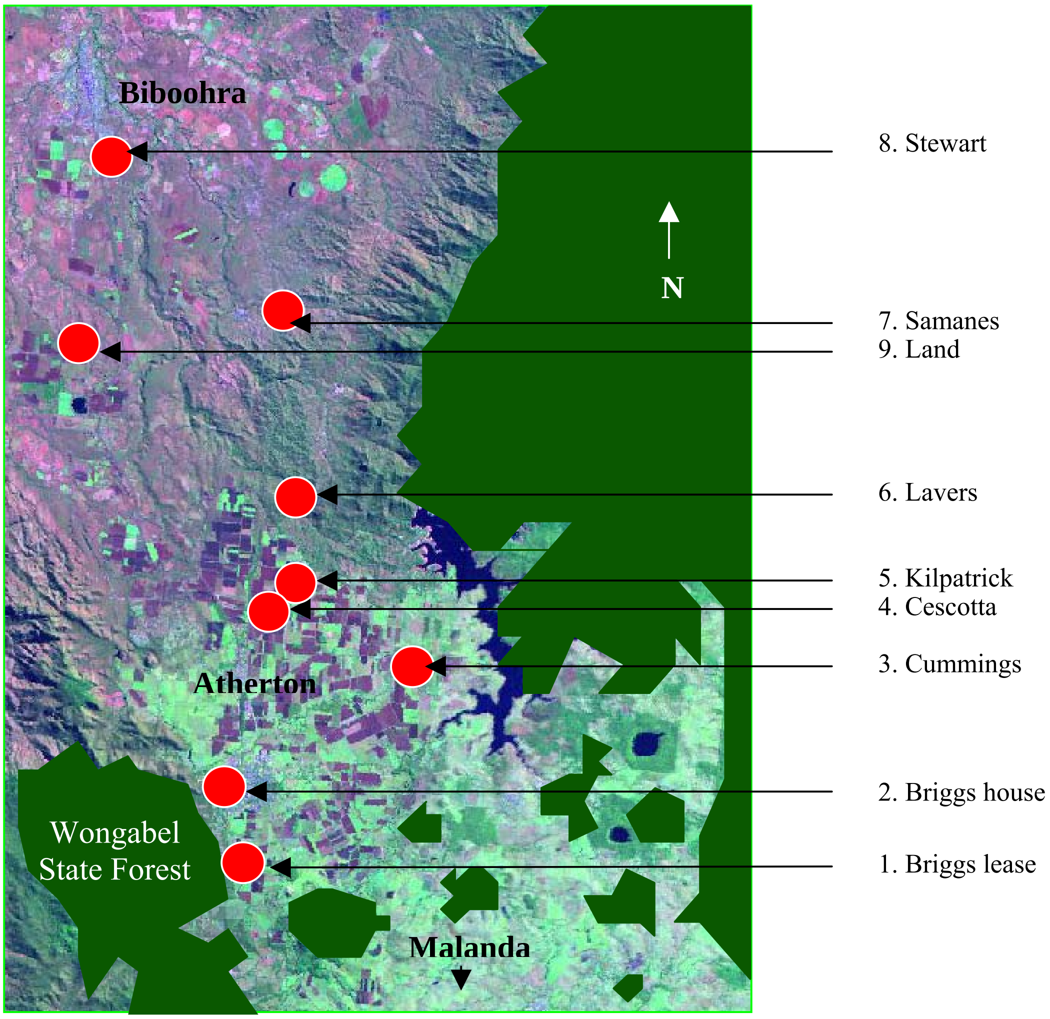

Figure 2.3 Location of the custard apple orchard study sites on the Atherton Talelands, north Queensland and their approximate distances from rainforest habitat (not to scale).

The orchards were chosen to be at varying distances from rainforest habitat to enable the comparison of pollinator abundances and fruit productivity against the continuous variable, distance to nearest rainforest.

Orchard 1 (Briggs' Lease) is located 100 m from rainforest, orchard 2 (Briggs' House) is located 400 m from rainforest habitat, orchard 3 (Cummings) is located 500 m from rainforest, orchard 4 (Cescotta) is located 5 km from rainforest, orchard 5 (Kilpatrick) is located 5.5 km from rainforest, orchard 6 (Lavers) is located 9 km from rainforest, orchard 7 (Samanes) is located 12 km from rainforest, orchard 8 (Stewart) is located 16 km from rainforest and orchard 9 (Land) is located 24 km from rainforest habitat.

The configuration of increasing distance from rainforest followed the gradient of decreasing rainfall between Malanda and Mareeba (Table 2.1). Thus, the potential for a significant cross correlation between the distance to nearest rainforest habitat and mean annual rainfall exists in the spatial design of the study. This was unavoidable due to the position of available orchards in space. Where appropriate the potential association between distance and rainfall are considered in the interpretation of results.

Table 2.1. Mean annual values for the climate parameters rainfall and temperature from the nearest town centres to orchard sites (nb. Tolga temperatures are a min. max. range) (Adapted from Commonwealth Bureau of Meteorology and Malcolm *et al* (1999)).

Orchard site	*Nearest centre for climate record*	*Mean annual rainfall (mm)*	*Temperature (^{o}c)*		*Relative position across the tablelands*
			Mean max.	*Mean min.*	
Briggs Lease	Atherton	1413.4	26	14.5	Southeast
Briggs House	Atherton	1413.4	26	14.5	
Cummings	Kairi	1279	25.2	15.6	
Cescotto	Tolga	1350	29.4-21.2	18.3-10.0	
Kilpatrick	Tolga	1350	29.4-21.2	18.3-10.0	
Lavers	Walkamin	1034.9	27.2	17	
Samanes	Mareeba	925.1	28.8	16.1	
Stewart	Biboohra	925.1	28.8	16.1	Northwest
Land	Dimbulah	790.7	31.5	16.6	

2.3. Study species

The study species is the commercially grown custard apple. In Australia this is a hybrid between two species, *Annona squamosa* L. and *Annona cherimola* Mill. within the basal angiosperm family Annonaceae. *Annona squamosa* originates in the American tropical lowlands and *Annona cherimola* from the highlands of Equador and Peru (Shroeder 1995). The hybrid cultivar is grown for fruit production in a number of countries outside either species' indigenous centres, including Australia, Spain, Israel and the United States. On the Atherton Tablelands, the main variety of custard apple grown for commercial fruit production is the Hillary White cultivar.

The custard apple is a small semi-deciduous tree that produces solitary flowers in the axils of leaves. Flowers are specialised into a floral chamber with three fleshy white/green petals enclosing many sex organs (Figure 2.4a). Flowers are hermaphroditic and protogynous, that is, both male and female reproductive structures are produced within the same flower although female and male reproductive maturity is temporally separated. The female reproductive structures mature first and can remain receptive for up to twenty-four hours (Gazit *et al.* 1982). Flowers then begin to dehisce and shed pollen and the petals open outwards revealing the sex organs (Figure 2.4b.).

Protogyny is considered to be a mechanism to ensure out-crossing and reduce self-fertilisation within the same flower (Thakur & Singh 1965). However, self-fertilisation does occur in custard apple flowers, particularly when humidity is high.

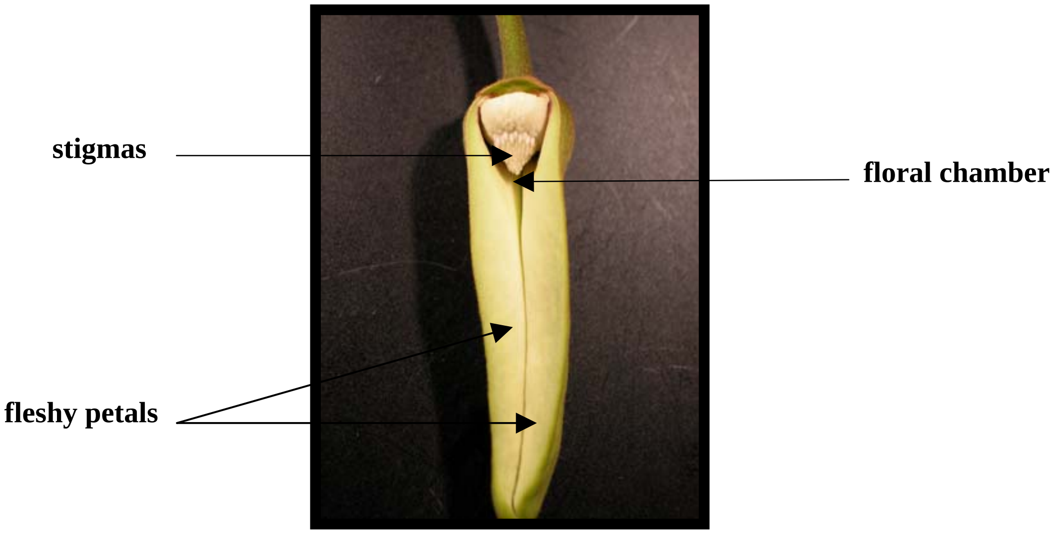

Figure 2.4a. Longitudinal section through an *Annona squamosa* x *Annona cherimola* (Family Annonaceae) flower in the receptive female stage.

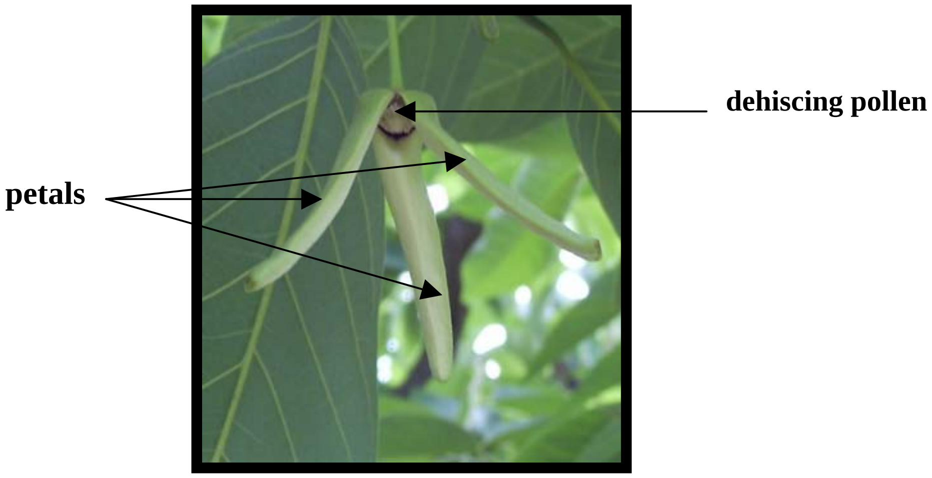

Figure 2.4b. ***Annona squamosa*** **x** ***Annona cherimola*** **(Family Annonaceae) flower in the male stage.**

Self-fertilisation has only been recorded in custard apple flowers, when humidity exceeds 70% (George *et al* 1989). When the humidity is greater than 70%, the stigmatic surface during the female stage remains moist, and therefore receptive; at the onset of anther dehiscence (male reproductive maturity) and pollen can be transferred between the anthers and stigmas of the same flower (George *et al* 1989). Even during periods of high humidity, when self-fertilisation has been recorded, the percent fruit set that can be attributed to self-fertilisation in the absence of biotic pollinators is extremely low relative to fruit set in flowers visited by beetles. For example, George *et al* (1989) recorded 5.7% fruit set in flowers that were open pollinated compared with 1.4% and 1.5% fruit set in flowers that were enclosed in paper bags and gauze bags respectively to prevent biotic pollinator visits. Custard apple reproduction appears to be autogamous-allogamy (Richards 2001). This means that flowers are self-fertile, although, visits by animal pollinators transferring outcrossed pollen significantly increases fruit quantity and quality (Gazit *et al* 1982, George *et al* 1992).

Custard apples are beetle pollinated (cantharophilous), predominantly by beetles in the family Nitidulidae (sap beetles) (Gazit *et al* 1982, George *et al* 1992). Other beetle families may also be involved in pollination. On the Atherton Tablelands, beetles from the families Mycetophagidae (*Litargus* sp.) and Lathridiidae (*Lathridiid* sp.) have been recorded visiting female flowers in custard apple orchards (Blanche *et al.* unpublished) and there is some circumstantial evidence that Staphylinid beetles, *Coccinella* sp. and *Scymnus* sp. (Coccinellidae), thrips and possibly an *Orius* bug may be responsible for pollination in other countries such as Spain and the United States (Ahmed 1936, Schroeder 1995). Flowers produce a strong fruity or aminoid floral odour during both the male and female reproductive phase, which is proposed to be important in long distance beetle attraction (Montieth 1973). In the case of custard apple flowers, beetles are thought to be attracted to the fruity or aminoid floral odour by deceit (Gottsberger 1974). This is because the floral scent imitates the odour of decaying fruit and many of the larvae of species that pollinate custard apple flowers feed on decomposing fruit (Nadel 1990). The chamber blossom could provide a protective place where pollinators feed, copulate or lay eggs (Gottsberger 1974) (Figure 2.4a), however, the activities of pollinators within custard apple flowers are still unknown.

Productivity (quantity and quality of fruit) in commercial custard apple orchards is typically low relative to the number of flowers available for fertilization. Low productivity has been attributed to pollinator limitation and physiological stress (Shroeder 1995, Pẽna *et al* 1999). Pollinator limitation is implied because the probability of initiating a fruit is positively correlated with the number of beetles within each female flower (George *et al.* 1989) and fruit initiation and quality (mass and symmetry) is significantly improved when pollen is added manually to receptive stigmas (Schroeder 1995).

In many commercial growing areas hand-pollination is required to increase crop productivity and maintain orchard viability. Hand-pollination involves the manual addition of newly shed pollen from one flower to the receptive stigmatic surfaces of another flower. George *et al* (1989) found that the proportion of flowers that set fruits increased from 5.7% in open pollinated flowers to 27.6% in flowers that were also hand pollinated. The success of this technique varies considerably among flowers causing fruit initiation and fruit quality to be highly variable on a single tree (Briggs pers. comm.). There are a number of potential explanations for this variability. In many out-crossing crop cultivars, the quality of pollen can determine the probability of fruit initiation, fruit maturation and resultant fruit quality (Wallace & Lee 1999, Wallace *et al* 2002). In commercial custard apple orchards, trees of the African Pride cultivar are maintained in the orchard solely as a source of pollen for augmenting female flowers during hand-pollination. Pollen from both the African Pride cultivar and the Hillary White cultivar is used for hand-pollination but only female flowers of the Hillary White cultivar are hand-pollinated. Therefore, the source of pollen used in hand-pollination may be a factor that influences fruit production in this crop.

Chapter 3

Species diversity and abundance of potential pollinator species decreases with increasing isolation from remnant rainforest habitat.

Abstract

Biotic pollinators initiate or enhance fruit and seed production in at least 90% of angiosperms including native and agricultural species. The ubiquitous honeybee has assumed the role of dominant pollinator in crop situations; however, honeybee colonies are suffering increased mortality in all bee-keeping countries except Australia (Cunningham et al 2003, Kearns et al 1998). It is becoming clear that agricultural sustainability depends on finding alternative pollinator species to provide sufficient pollination services to crops either as managed or wild foraging species (Heard 1999, Allen-Wardell et al 1998). Maintaining sufficient populations of wild pollinator species or assemblages in a landscape is only possible with an understanding of the resources or elements of a landscape that they require (Allen-Wardell et al 1998). I have surveyed floral visitors at nine commercial custard apple (*Annona squamosa* x *A. cherimola*) positioned at increasing isolation from naturally occurring rainforest. I found 19 species of potential pollinators (Nitidulidae, Mycetophagidae, Lathridiidae, Staphylinidae, Curculionidae, Rhizophagidae, Anthicidae (beetles), Thysanoptera (thrips), 16 of which may be new records for custard apple pollination. The results show that fewer species and individuals of floral visitors are in custard apple orchards isolated from naturally occurring rainforest. Some species only occurred in orchards within 400 metres from rainforest. This indicates that these species are likely to use, or require, rainforest resources in the

landscape and are limited in their ability to disperse to isolated orchards. Although there was a weaker relationship between floral visitor abundance and species richness and rainfall it is not unlikely that rainfall is a limiting factor associated with this gradient in the landscape whether it is in isolation from or combination with crop isolation to naturally occurring rainforest.

Introduction

Managed and non-managed invertebrate and vertebrate fauna provide biotic pollination services to crop plants (Free 1993). In temperate agriculture in particular, pollination services for most crop species have traditionally been provided primarily by managed honeybees (*Apis mellifera*).

There are numerous crop species for which honeybees are less effective or ineffective pollinators, however (Free 1993, Kenmore & Krell 1998). It has been estimated that only 15% of the 100 most important crop species are pollinated by domesticated bee species, which includes honeybees, compared to 80% that are pollinated by wild bee species (Kenmore & Krell 1998). Furthermore, future reliance on honeybees for pollination services may be untenable. Managed honeybee colonies have suffered increased mortalities due to infestations of parasitic mites in all bee-keeping countries (except Australia), raising concern over the future viability of managed honeybee populations. Thus, the ability of honeybees to provide sufficient pollination services to crops into the future is uncertain (Cunningham *et al* 2003, Kearns *et al* 1998).

In light of the potential decline of honeybee services, and their unsuitability for pollinating particular crop species, more emphasis has been directed to identifying alternative pollinators that may be effective pollinators in commercial crop situations (Heard 1999, Allen-Wardell *et al* 1998). The majority of potential pollinators are likely to be arthropods as they comprise the largest proportion of the pollinator fauna for crops therefore most work has been directed to these groups (Free 1993). In particular, emphasis has been placed on identifying all species that either currently provide some unmeasured contribution to pollination services, or have the potential to do so (Heard 1999). Potential pollinators are a subset of all species occurring in areas wherever crops are grown. Because crop cultivation transports plant species outside of their centre of origin, the potential pollinators include species with wide (cosmopolitan) geographic distributions that may or may not have been transported with the crop species, as well as other species that are endemic to the area where cultivation takes place and not normally associated with the crop species.

In instances where alternative pollinators have been identified, research has concentrated on exploring the potential to manage alternative species via one of two different methods (Heard 1999, Bosch & Kemp 2002). One method attempts to enhance alternate pollinator populations within orchards themselves (George *et al*1992, Peña *et al* 1999). For example, in custard apples species-specific attractants (fruit and pheromones) are used to increase sap beetle (Nitidulidae) populations within orchards. The second method attempts to identify the habitat requirements of native species present in the landscape and to initiate and implement plans to maintain these landscape elements. This ensures pollinators arrive at orchards in sufficient numbers and provide viable pollination services (Allen-Wardell *et al* 1998). This later strategy is of particular interest, because services provided by endemic species can be used to assess the economic value of retaining remnant natural or semi-natural habitat patches in the landscape, providing landholders with incentive to actively conserve natural vegetation (see Chapter 1).

Landscape-level distribution of requirements of potentially important native pollinators species is particularly important to understand (Allen-Wardell *et al* 1998).

In agricultural regions, landscapes are typically a matrix of land under cultivation and remnant native habitats. Modern farming areas usually contain single plant species (monoculture) stands surrounded by agriculture or infrastructure, or sharply delineated from adjacent native habitats by a well-defined edge or boundary (Altieri 2000). Arthropod species diversity within monocultures is known to be significantly lower than it is in floristically diverse habitats such as intact native habitats or complex traditional agricultural crop stands that contain alternative hosts, host plants and nesting sites required for arthropod lifecycles (Perfecto *et al* 1997, Thies *et al* 2003). For example, in Costa Rica, Perfecto *et al* (1997) found that beetle diversity was 59% and ant diversity 78% lower in a monoculture stand of coffee (*Coffea arabica*) compared with that of an intact tropical forest or a traditional plantation containing a number of species of shade trees (ie. *Erythrna poeppigiana*, *Erythrina fusca*, *Annona cherimoya*) interspersed among coffee trees.

Because of the paucity of faunal species in modern agricultural crops, species rich, remnant native habitats adjacent to crop areas are often a ‘source’ of beneficial invertebrates, including pollinating species, to adjacent crop areas (Heard & Exley 1994, Steffan-Dewenter & Tscharntke1999, Kremen *et al* 2002, Steffan-Dewenter *et al* 2002). The distribution of these ‘source’ areas in a landscape has been found to influence the composition and abundance of a pollinator fauna visiting crop plants. For example, social bee diversity and abundance visiting flowers was negatively related to the distance a crop was located from remnant calcareous grassland in Germany (Steffan- Dewenter & Tscharntke 1999) and native bees are positively related to the proportion of natural habitat near farms in California (Kremen *et al* 2004).

In Australia, very little is known about pollination services provided by native species; this is particularly true for tropical horticultural crops. Furthermore, few studies have established whether landscape structure (crop isolation from native habitat) determines the species richness and abundance of potential pollinators visiting crop flowers. In this chapter I identify the potential pollinators of custard apple (*Annona squamosa* x *A. cherimola)*, a tropical horticultural crop in north Queensland. Custard apples are tropical species and known pollinators from other growing regions are arthropods (particularly beetles). The most likely location of potential pollinators is rainforest, which are known to have high abundance and diversity of arthropods (Perfecto *et al* 1997). Therefore, I also examine the relationship between floral visitor species richness and abundance with respect to isolation of custard apple crops from naturally occurring rainforest habitat. If floral visitor species richness and abundance is negatively associated with distance from naturally occurring habitat as suggested by Steffan- Dewenter & Tscharntke (1999), then this would be strong evidence that floral visitors originate from these areas. More importantly, this evidence could be used to initiate widespread recognition of the services provided by high diversity habitats in agricultural landscapes as a means of arguing for their retention. In this chapter I have also tested the relationship between floral visitor species richness and abundance and average yearly rainfall data because this factor is correlated with the change in orchard isolation with distance from naturally occurring rainforest and the strength of the relationship could determine the most influential factor.

Methods

To assess the diversity and abundance of arthropods in custard apple orchards and the relationship with isolation, nine custard apple orchards located at increasing distance from naturally occurring rainforest were chosen for sampling arthropod visitors to flowers (Table 3.1). Sampling was carried out between October and December 2002 on the Atherton Tablelands, north Queensland (see Chapter 2).

Table 3.1. Custard apple (*Annona squamosa* x *A. cherimola*) orchards used for sampling floral visitors and their distance to naturally occurring rainforest.

Farm	Distance to naturally occurring rainforest (km)
Briggs Lease	0.1
Briggs House	0.4
Cummings	0.5
Cescotto	5
Kilpatrick	5.5
Lavers	9
Samanes	12
Stewart	16
Land	24

In each orchard, 100 female stage ‘Hillary White’ cultivar flowers were collected during October 2002. Because orchards varied in the number of trees they contained, and I wanted equal representation of flowers between trees within orchards, the number of flowers sampled per tree was determined by counting all Hillary White cultivar trees and dividing this into 100 (Table 3.1). Thus, within each orchard I sampled the same number of flowers per tree, but between orchards the total number of trees available determined the number of flowers per tree that were sampled. All flowers were collected at 9am because previous studies have shown this to be the peak period of female receptivity and the highest numbers of pollinators are found in the chamber blossoms at this time (Nadel and Peña 1994) (For full description of flower form and function, see Chapter 2).

Each flower was placed in an individual vial containing 8 parts ethanol: 2 parts glycerol. This mixture preserves arthropods present in the flowers (Kearns & Inouye 1993). Each flower was examined for arthropods and all specimens initially sorted to morphospecies. Representatives of each morphospecies were then curated (dry mounted). Samples of each morphospecies were identified to lowest taxonomic level possible and were then sent to the Australian Museum for further identification. Because the taxonomy of arthropods is so poorly known, not all individuals could be identified to species. All arthropods that could be identified to species were classified as either “native” (only known from north east Queensland rainforest) or “exotic” (species who originate outside of Australia) based on expertise provided by David Britton (Australian Museum). Where full species identification was not possible, it was impossible to determine distributional status as native or exotic. This was the case for many specimens (Britton, Aust. Museum, pers. com.).

The period of sampling was an unusually dry year and arthropod abundance is known to be sensitive to rainfall (Blanche *et al* 2001). Because of this, abundances of floral visitors in orchards were very low relative to expectation based on results of a similar study by Blanche in 2001. Therefore, I sampled floral visitors again during December after the onset of rain. In each Orchard, I sampled 50 female stage flowers at 9 am and 50 male stage flowers at 5pm. In all other regards samples from the December collection were treated in an identical manner as described above.

Analysis

To examine the relationship between both arthropod species richness and arthropod abundance and distance from naturally occurring rainforest, I pooled the results of both sampling periods and counted the total number of species and the number of individuals per orchard. Sampling periods were pooled because overall numbers were small.

Regression analysis was used to examine the relationship between species richness and distance to naturally occurring rainforest and again for species abundance and distance to naturally occurring rainforest.

Because I was interested in proportional changes, I transformed both measures to their natural logarithm and fitted a straight-line relationship between them. In this case, where the estimated slope in log-log space cannot be distinguished from unity then the relationship is isometric (meaning the two measures scale proportionally). Significant deviations from unity, either positive or negative, indicate exponential increase or decrease respectively. I tested for deviation from unity by determining whether the 95% confidence interval for the estimated regression slope included 1.

This transformation also had the additional benefit of eliminating the need to fit a curvilinear model.

I performed this analysis at a number of levels. The analysis was first performed for all species combined (including all species of “native”, “exotic” and unknown distribution). To examine the potential for natural rainforests to provide potential pollinators that are “native” to north Queensland, I performed the same analysis using all species except those with known “exotic” distribution. Unfortunately the total number of species that were known “natives” was too small to separate analysis.

I also performed a regression analysis between the natural logarithm of rainfall for the nearest township to the orchard (see Chapter 2) and the natural logarithm of both total beetle abundance and species richness for all species. I am unable to separate rainfall and distance to nearest naturally occurring rainforest, however, this analysis can determine which factor produces the strongest relationship.

All analyses were performed using SPSS Ver.12 software (SPSS, Chicago, IL, USA)

Results

I collected a total of nineteen species of arthropods that were floral visitors to custard apple flowers from the nine orchards. Eighteen of these species were beetles belonging to the families Nitidulidae (9 species), Mycetophagidae (1 species), Anthicidae (2 species), Lathridiidae (1 species), Curculionidae (1 species), Rhizophoridae (1 species), Staphylinidae (2 species) and one unknown family; one species was a thrip (Thysanoptera). Three species were known cosmopolitans and three known natives. The remaining thirteen species could not be identified to species and therefore are of unknown origin (Table 3.2).

Table 3.2. Arthropods surveyed from 200 female custard apple cu. 'Hillary White' flowers in each of 9 orchards located across the Atherton Tablelands.

* cosmopolitan species.

** native species.

ORDER	FAMILY	GENUS	SPECIES
COLEOPTERA (beetles)	**NITIDULIDAE**	*Brachypeplus*	*Instriatus***
		Carpophilus	*hemipterus**
		Carpophilus	*maculatus**
		Carpophilus	*convexiusculus**
		Carpophilus	sp 1
		Carpophilus	sp 2
		Carpophilus	sp 3
		Unknown	sp 1
		Unknown	sp 2
	LATHRIDIIDAE	*Lathridiid*	sp.**
	MYCETOPHAGIDAE	*Litargus*	sp.**
	CURCULIONIDAE	Unknown	sp 1
	RHIZOPHAGIDAE	Unknown	sp 1
	STAPHYLINIDAE	Unknown	sp 1
		Unknown	sp 2
	ANTHICIDAE	Unknown	sp 1
		Unknown	sp 2
	UNKNOWN	Unknown	sp 1
THYSANOPTERA (thrips)		Unknown	sp 1

When all species collected at each orchard were pooled together, there was a significant negative relationship between the number of species visiting custard apple flowers and distance the orchard was located from naturally occurring rainforest (R^2 = 0.57, $F_{1,7}$ = 9.27, P = 0.01, 95% CL (b) = -0.35, - 0.04; Figure 3.1). More species were collected visiting custard apple flowers in orchards located in proximity to naturally occurring rainforest.

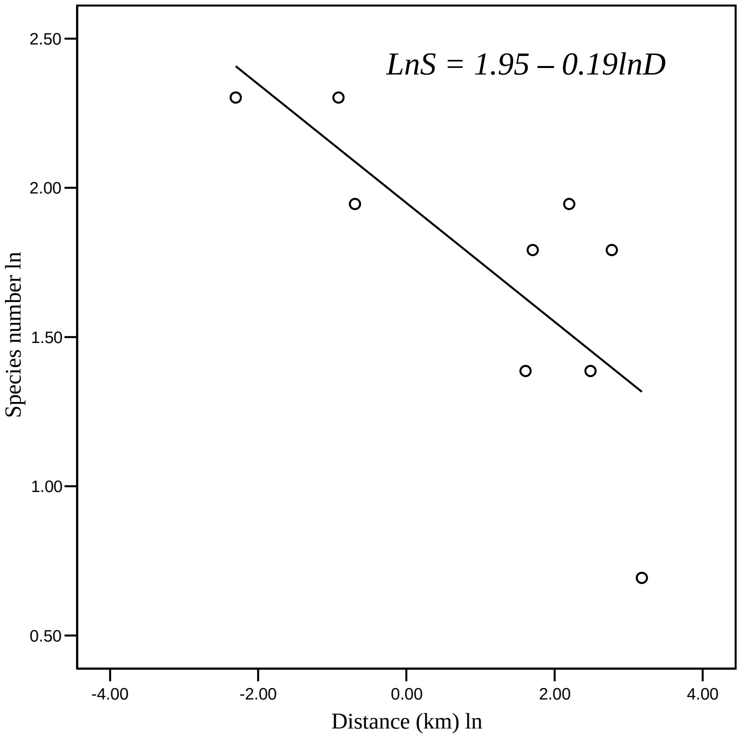

Figure 3.1. Relationship between the natural logarithm of floral visitor species number to 200 cu. 'Hillary White' custard apple flowers and the natural logarithm of distance the orchard was located to naturally occurring rainforest.

Similarly, the total abundance of all floral visitors within each orchard was negatively related to distance the orchard was located to naturally occurring rainforest (R^2 = 0.573, $F_{1,7}$ = 9.39, P = 0.018, 95% CL(b) = -0.76, -0.09; Figure 3.2); total arthropod abundance within a single orchard declined with distance from naturally occurring rainforest.

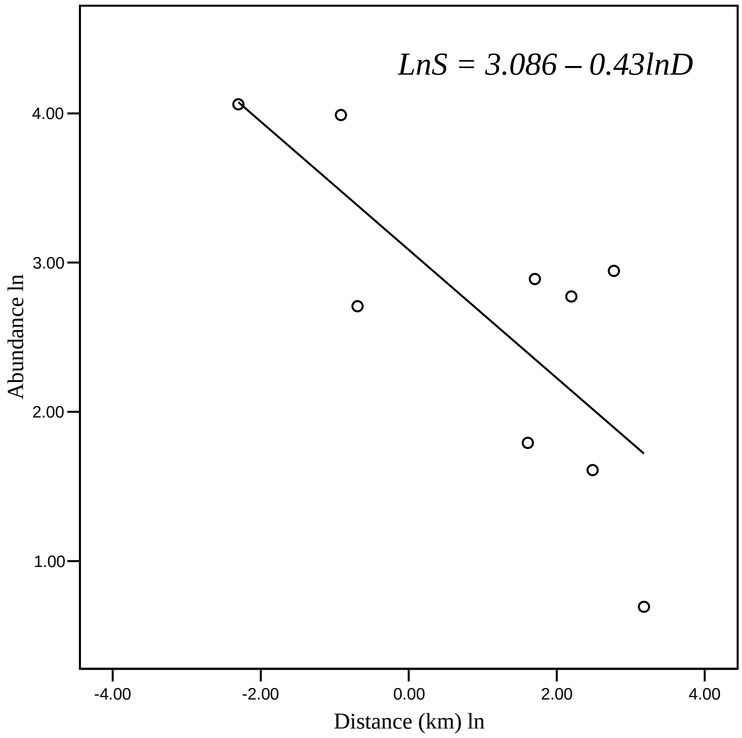

Figure 3.2. Relationship between the natural logarithm of total floral visitor abundance visiting 200 cu. 'Hillary White' custard apple flowers and the natural logarithm of distance the orchard was located to naturally occurring rainforest.

When known exotic species were excluded from the analysis, there was a significant negative relationship between the number of species visiting flowers and the distance an orchard was located to naturally occurring rainforest ($R^2 = 0.50$, $F_{1,7} = 7.09$, $P = 0.032$, 95% CL(b) = -0.33, -0.02; Figure 3.3). There was again a significant negative relationship between arthropod abundance (excluding exotic species) and distance to naturally occurring rainforest ($R^2 = 0.57$, $F_{1,7} = 9.39$, $P = 0.018$, 95% CL(b) = -0.76, -0.09 Figure 3.4). The proportion of the variability in each of the response variables by distance only changed marginally when known exotic species were excluded from the analyses.

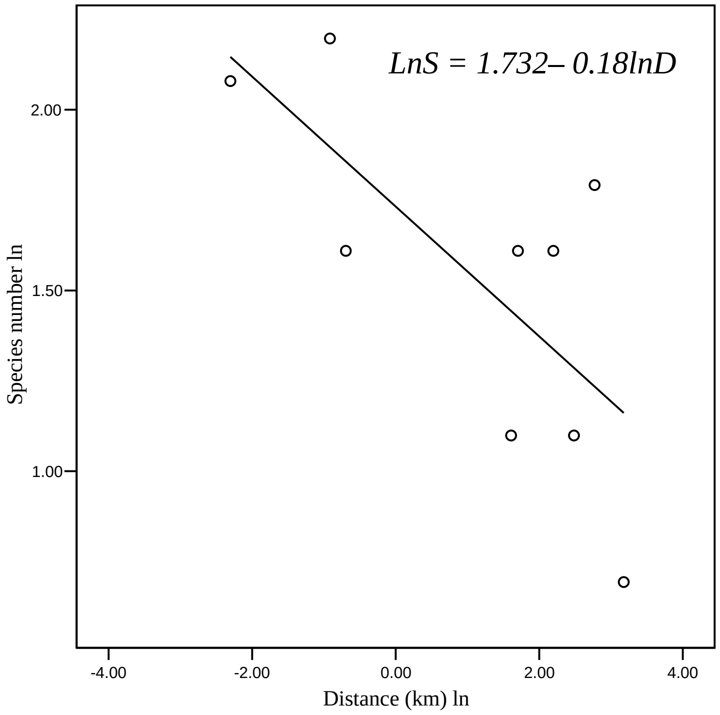

Figure 3.3. Relationship between the natural logarithm of the number of species of arthropod floral visitors (excluding known 'exotic' species) and orchard distance to naturally occurring rainforest in 200 'Hillary White' cultivar custard apple (*Annona squamosa* x *A. cherimola*) flowers in each orchard.

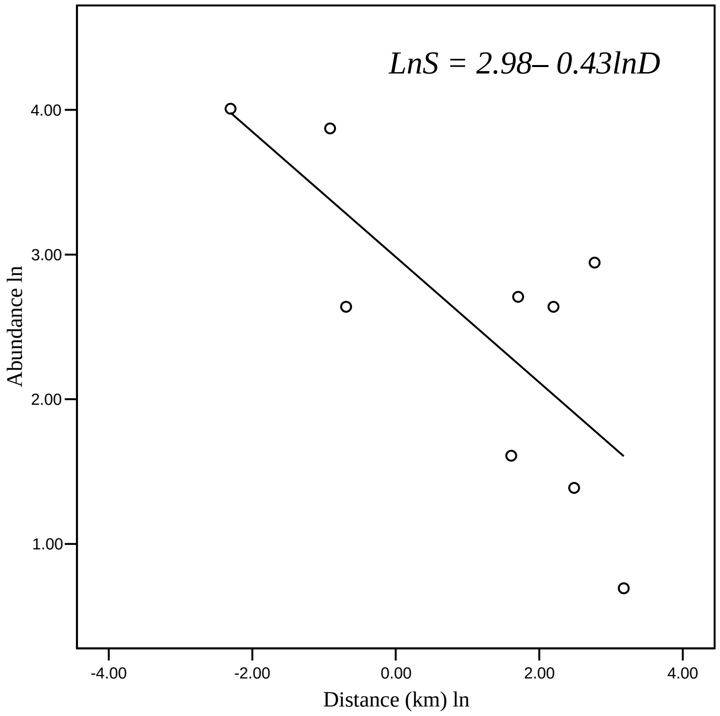

Figure 3.4. Relationship between natural logarithm of floral visitor abundance (excluding known 'exotic' species) and orchard distance to naturally occurring rainforest in 200 'Hillary White' cultivar custard apple (*Annona squamosa* x *A. cherimola*) flowers in each orchard.

When I examined rainfall as a potential determinant of floral visitor abundance and species richness for all species there was a significant relationship in both cases (Species abundance $R^2 = 0.5$, $F_{1,7} = 7.13$, $P = 0.03$; Species richness $R^2 = 6.62$, $F_{1,7} = 6.62$, $P = 0.04$), however, the relationship is not as strong as that with distance to nearest naturally occurring rainforest.

Discussion

Here I have presented evidence that species richness and abundance of arthropods that potentially provide pollination services to custard apple crops declined significantly and exponentially with crop isolation from naturally occurring rainforest. This is some of the first evidence of an effect of landscape structure on a pollinator community visiting a crop both within the tropical Australian landscape and for a group of arthropods other than native social bees (see Blanche & Cunningham 2005); all arthropod floral visitors in this survey were beetles and a thrip (Coleoptera and Thysanoptera) (Steffan-Dewenter & Tscharntke 1999, Ricketts *et al* 2004).

Eighteen of the total nineteen species of arthropods sampled from female custard apple flowers were beetles (Coleoptera) comprising eight families and one species of thrip (Thysanoptera). Arthropods were identified to the lowest taxonomic level and determined as native, exotic or unknowns and when all groups were treated together, and then excluding known exotic species, I found significant negative relationships between both species richness and arthropod abundance and crop distance from naturally occurring rainforest. All relationships were non-linear; arthropod species richness and abundance declined exponentially with distance from naturally occurring rainforest.

These results indicate that all the arthropods surveyed in this study are at least partially dependent on adjacent naturally occurring rainforest and are likely to derive from this habitat to forage in custard apple crop flowers. The potential confounding effect of a rainfall gradient must also be considered here however. Beetle abundance is known to be influenced by rainfall (Blanche *et al* 2001) and these two factors could not be isolated in this study due to a lack of replicated orchards at various distances from naturally occurring rainforest. It is therefore important to identify the potential for rainfall to be a significant determinant of arthropod visitors across the landscape, potentially as a co-factor with crop isolation although it appears from the strength of the relationships in this study and Blanche & Cunningham (2005) that crop isolation is still an important factor operating in this system.

There were few species that could be identified further than family taxonomic level and only three of these are known natives (*Lathridiid* sp., *Litargus* sp. and *Brachypeplus instriatus*). Nine species of beetles of the family Nitidulidae were collected visiting custard apple flowers; one native species (*Brachypeplus instriatus*), five species that were unable to be identified and have the potential to be native species and three exotic species (*Carpophilus hemipterus, C. maculatus, C. convexiusculus*); *Carpophilus* spp. are known pollinators of custard apples in other commercial growing regions (George *et al* 1992, Peña *et al* 1989).

Beetles of the family Nitidulidae are well known pollinators of custard apples in other growing regions and have also been recorded pollinating other plant species in the family Annonaceae (Gazit *et al* 1982, George *et al* 1989, Nagel *et al* 1989, George *et al* 1992, Gottsberger 1999, Nadel & Peña 1994, Peña *et al* 1999, Silberbauer-

Gottsberger *et al* 2003). Annonaceae is a primitive angiosperm family that is well represented in Australian rainforests (32 species) (Hyland *et al* 1999). A number of the Australian rainforest Annonaceae contain a similar floral form to custard apples producing fleshy petals that often form a floral chamber, produce a stigmatic exudate and a strong floral odour which is known to be important in long distance beetle attraction (Monteith 1973). Pollination studies of Annonaceae (*Pseudovaria* spp.) in north Queensland rainforests have found that native beetles from the family Nitidulidae are the most common pollinators (Hill 2003). The nitidulid beetles recorded in this survey are therefore likely to be pollinating custard apple flowers and could derive from rainforest habitat where they could contribute to pollination in other plant species within the family Annonaceae.

Thysanoptera (thrip), Curculionidae (weevils), Rhizophagidae and Anthicidae (beetles) are new records for custard apple floral visitors. Thrips and weevils (Curculionidae) have been recorded pollinating other Annonaceae (Gottsberger 1999, Silberbauer-Gottsberger *et al* 2003). Species from these families were only collected in custard apple orchards located within 400 metres from naturally occurring rainforest indicating the potential for them to be rainforest-derived species. Thrips are known pollinators of other species of Annonaceae, for example *Bocageopsis multiflora* and *Oxandra euneura* (Webber & Gottsberger 1995) and as floral visitors carrying pollen in *Pseudovaria* spp. (Hill 2003). Thrips are often overlooked as effective pollinators in species that they visit and when occurring in high numbers have been shown to make an effective contribution to fruit production (Terry 2001).

‘Rove’ beetles (Staphylinidae) have been recorded visiting custard apple flowers in Spain (Ahmed 1936) and as pollinators of other Annonaceae (Gottsberger 1999, Silberbauer-Gottsberger *et al* 2003). The number of individuals of staphylinid beetles was quite low (n = 4), however, species 1 was only recorded in the orchards within 400 metres from naturally occurring rainforest and may therefore derive from rainforest and contribute to pollination in these orchards.

The species and origin of the beetles within Rhizophagidae and Anthicidae were undetermined, however, there are no records indicating that Rhizophagidae beetles are pests within the Australian landscape and therefore it is likely that the species collected in this survey is of native origin. Interestingly, little is known about the biology of these beetle groups and no records at all could be found for these groups containing pollinating species, despite the common name given for Anthicidae (“ant-like” flower beetles). The species collected in this survey cannot be ruled out as potential pollinators because they have a body form characteristic of other custard apple pollinating beetle species including small size (3mm or less) and dense hairs covering the body that is a known trait required, and likely, to trap pollen grains.

Beetle pollinators comprise a large proportion of the Australian insect fauna, at least 28 of the 121 beetle families contain anthophilous (flower visiting) species and beetle diversity is high in Australian tropical rainforests; including species that are known pollinators of rainforest trees (eg. *Alphitonia petriei*) (Irvine & Armstrong 1988). Because of the representation of other beetle and thrip pollinated Annonaceae and the high diversity of arthropod species (including pollinators) in Australian rainforests, it is likely that a proportion of the floral visitors found in this study are native species deriving from adjacent naturally occurring rainforest. These results combined with empirical research from other regions demonstrate that crop isolation influences the distribution of a number of pollinating agents including social bees, beetles and thrips and most likely many other crop pollinating groups deriving from, or partially dependent on, adjacent native habitats.

Chapter 4

Inferior biotic pollination services in custard apple orchards isolated from naturally occurring rainforest.

Abstract

Landscape structure (composition and arrangement of native vegetation and agricultural land at intermediate scale) has been shown to influence productivity in crop species such as Macadamia nut, mustard and radish. This is because the proportion of native habitat in a landscape or alternatively the isolation distance a crop is located from native habitat determines the composition and abundance of a pollinator fauna visiting those crops (Heard & Exley 1994; Steffan- Dewenter & Tscharntke 1999, Kremen *et al* 2002, Steffan-Dewenter *et al* 2002). I have measured fruit productivity resulting from self-pollination, biotic pollination and hand-pollination in custard apple (*Annona squamosa* x *A. cherimola*) orchards located at increasing isolation from naturally occurring rainforest. Here I present evidence that isolation distance between orchards and naturally occurring rainforest and fruit productivity are negatively related in beetle pollinated custard apple crops. It is cautioned however that rainfall could not be separated from crop isolation in this empirical study and is related to arthropod abundance and species richness and therefore has the potential to influence fruit productivity in these orchards.

No fruit was produced in flowers where biotic pollinators were excluded, indicating that they are required for fruit production, although, none of the orchards received enough pollen to maximise fruit set. All orchards were pollen limited, and produced almost 100 % fruit set when pollen was manually added to flowers, regardless of position in the landscape. However, orchards at proximity to naturally occurring

rainforest were less pollen limited than orchards in isolation from naturally occurring rainforest indicating that there are fewer pollinators servicing them.

Introduction

Landscape structure is a term that describes the relative proportion and configuration of different land uses across a continuous geographic region at intermediate spatial scale (Loreau *et al* 2003). Landscape structure has been identified as a critical factor determining productivity in biotically pollinated crops (Heard & Exley 1994; Steffan-Dewenter & Tscharntke 1999, Kremen *et al* 2002, Steffan-Dewenter *et al* 2002). This is because the configuration of native habitats and cultivated land determines the composition and abundance of the pollinator community visiting a crop (Chapter 3). The composition and abundance of a pollinator community influences fruit and seed production because the number of successful pollination events resulting in a fruit and/or seed is a function of both pollination rate (pollinator abundance) (Aizen & Feinsinger 1994, Cunningham 2000, Mustajarvi *et al* 2001, Goulson & Derwent 2004) and the efficiency of each pollinator species (pollinator composition) within the agricultural landscape (Motten *et al* 1981, Klein *et al* 2003).

Pollinators vary considerably in their ability to initiate seed set from a single visit (pollinator efficiency) (Motten *et al* 1981, Gross & Mackay 1998, Mayfield *et al* 2001, Thomson & Goodell 2001). This can be due to a pollinator species degree of fidelity to a single plant species that determines the proportion of con-specific pollen carried by an individual pollinator that is subsequently deposited on a stigma of the same species (Motten *et al* 1981). For example generalist pollinators may visit flowers of a number of plant species in a single foraging episode. In these cases, generalist pollinators have greater potential to transfer incompatible pollen between plant individuals, compared to pollinators that visit a single plant species only (Carthew 1993); although this is not always the case (see Motten *et al* 1981).

In addition, potential pollinator species may differ in their efficiency in removing and/or depositing con-specific pollen on the stigma of a receptive plant. These differences in pollen transfer and deposition efficiencies between pollinators arise due to differences in foraging behaviours on flowers and inflorescences (Carthew 1993, Gross & Mackay 1998, Mayfield *et al* 2001, Potts *et al* 2001, Thomson & Goodell 2001). For example, Carthew (1993) found that the pollinators of *Banksia spinulosa* (Proteaceae) varied both in their ability to remove pollen from pollen presenters and their frequency of contact with receptive stigmas. Small mammals (i.e. sugar glider: *Petaurus breviceps,* eastern pygmy possum: *Cercartetus nanus,* brown antechinus: *Antechinus stuartii*) were the most efficient pollinators because they removed pollen more often than bees (introduced and native), moths (Noctuidae) and birds (eastern spinebill: *Acanthorhynchus tenuirostris*) and were more likely to contact the receptive stigmatic surfaces because they moved all over the inflorescence (Carthew 1993). The combined influence of rate and efficiency means that the services provided by pollinator communities cannot be assessed via abundances alone (Klein *et al* 2003).

Therefore understanding the potential factors that influence the composition and abundance of pollinators will be of central importance in any attempts to explain the productivity of biotically pollinated crops within an agricultural landscape.

Isolation distance of a crop from native habitats has been demonstrated to be an important determinant of the composition and abundance of pollinator communities, and resulting crop productivity in fragmented agricultural landscapes (Steffan-Dewenter & Tscharntke 1999, Steffan-Dewenter *et al* 2002, Ricketts 2004, De Marco Jr, P. & F.M. Coelho 2004). For example, in Germany, Steffan- Dewenter &

Tscharntke (1999) found that there was a significant negative relationship between plant isolation from remnant calcareous grassland and the number of fruit and seeds produced per plant in both mustard (*Sinapis arvensis*) and radishes (*Raphanus sativus*); plant reproductive output (number of seeds per plant) declined by 50% at 260 metres from the grassland for radish (*R. sativus*) and 1000 metres from grassland for mustard (*S. arvensis*) plants. In that system, both mustard and radish are pollinated by bee species occurring naturally in neighbouring remnant calcareous grassland and the decline in crop productivity was associated with a significant decline in the abundance of wild bees with increasing isolation from the remnant calcareous grassland.

Crop isolation from native habitat is likely to be an important factor influencing crop productivity, particularly in crop species that are not effectively pollinated by managed honeybees (*Apis mellifera*), but also in those crops that are additionally serviced by native species. Crops that are not effectively pollinated by honeybees will be particularly vulnerable to low fruit productivity due to pollinator limitation because managed pollinator species are not able to replace a depauperate native pollinator community.

Custard apple (*Annona squamosa* x *Annona cherimola*: Family Annonaceae) is a crop that requires biotic pollination services to initiate and enhance fruit production. Fruit set is less than 1.5 % in the absence of pollinators (George *et al* 1989) and honeybees are not effective pollinators of this crop. In fact, honeybees could have a potentially negative influence on productivity in custard apple orchards because they rob flowers of pollen during the male reproductive stage (Schroeder 1995). Small beetles in the family Nitidulidae predominantly pollinate the flowers. Tropical rainforests contain a high diversity of beetle species and are likely to represent reservoirs of potential pollinators that are more diverse, and more numerous than an adjacent agricultural landscape (Perfecto *et al* 1997). Therefore, isolation from rainforest habitat, a potential source of beetle pollinators, could influence the composition and abundance of pollinator species in custard apple orchards and consequently the productivity (fruit initiation) of those crops.

Aims

The aim of this empirical research was to determine whether isolation by distance of custard apple orchards from naturally occurring rainforest had a negative influence on fruit production. I asked the following questions: (1) do custard apples self-pollinate? (2) How much fruit production can be attributed to biotic pollinators? (3) Does the quantity and / or quality of fruit produced via biotic pollinators decline with the distance of a crop from naturally occurring rainforest? (4) Is the difference in fruit production due to pollen limitation?

Methods

Orchard isolation from naturally occurring rainforest

Five orchards were selected to test for an effect of isolation from rainforest habitat (distance) on the productivity (fruit initiation) of the custard apple crop. Orchards were located at increasing isolation from rainforest habitat at: 100 metres (Briggs lease orchard), 500 metres (Cummings orchard), 5.5 kilometres (Kilpatrick orchard), 9 kilometres (Lavers orchard) and 12 kilometres (Samanes orchard) (Fig 4.1).

African Pride and Hillary White cultivar custard apples are grown in all the orchards. However, only the Hillary White variety was used for measuring productivity.

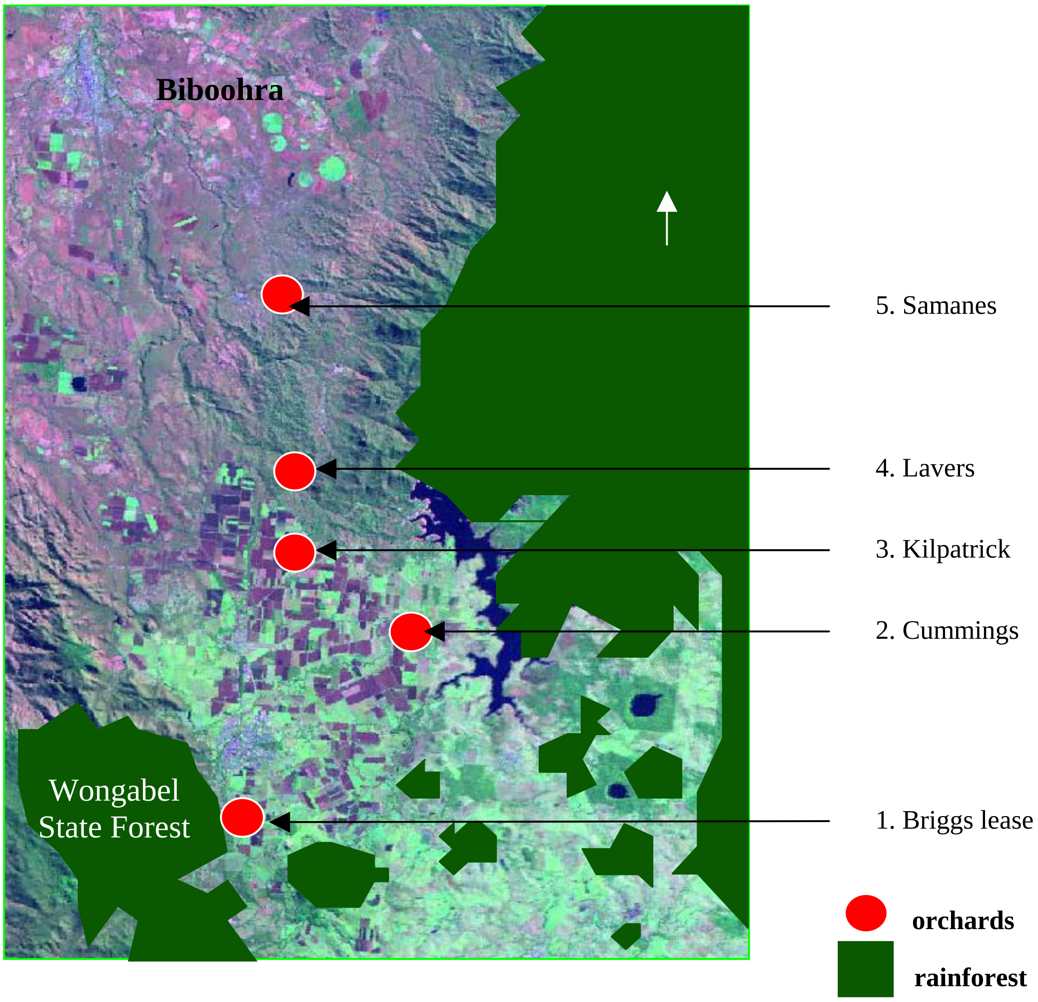

Figure 4.1. Location of the custard apple orchard sites that were used for pollination experiments. Orchard numbers are ascending in correspondence with the distance an orchard is located to nearest naturally occurring rainforest. Orchards are located across the Atherton Tablelands, north Queensland, Australia (not to scale).

Pollination experiments

Within each of the five orchards, all trees of the Hillary White cultivar were numbered and ten trees were selected using random number table. These trees were used for the experimental treatments. There were three treatments nested within each of the trees to eliminate a tree effect (a schematic representation of the experimental design in shown in Fig 4.2). The treatments were: 1) Pollinator exclusion, to measure fruit initiation due to self-pollination in the absence of biotic pollinators 2) Open pollination, to measure fruit set attributable to biotic pollination and self pollination and 3) Hand pollination, to test whether flowers are pollen limited.

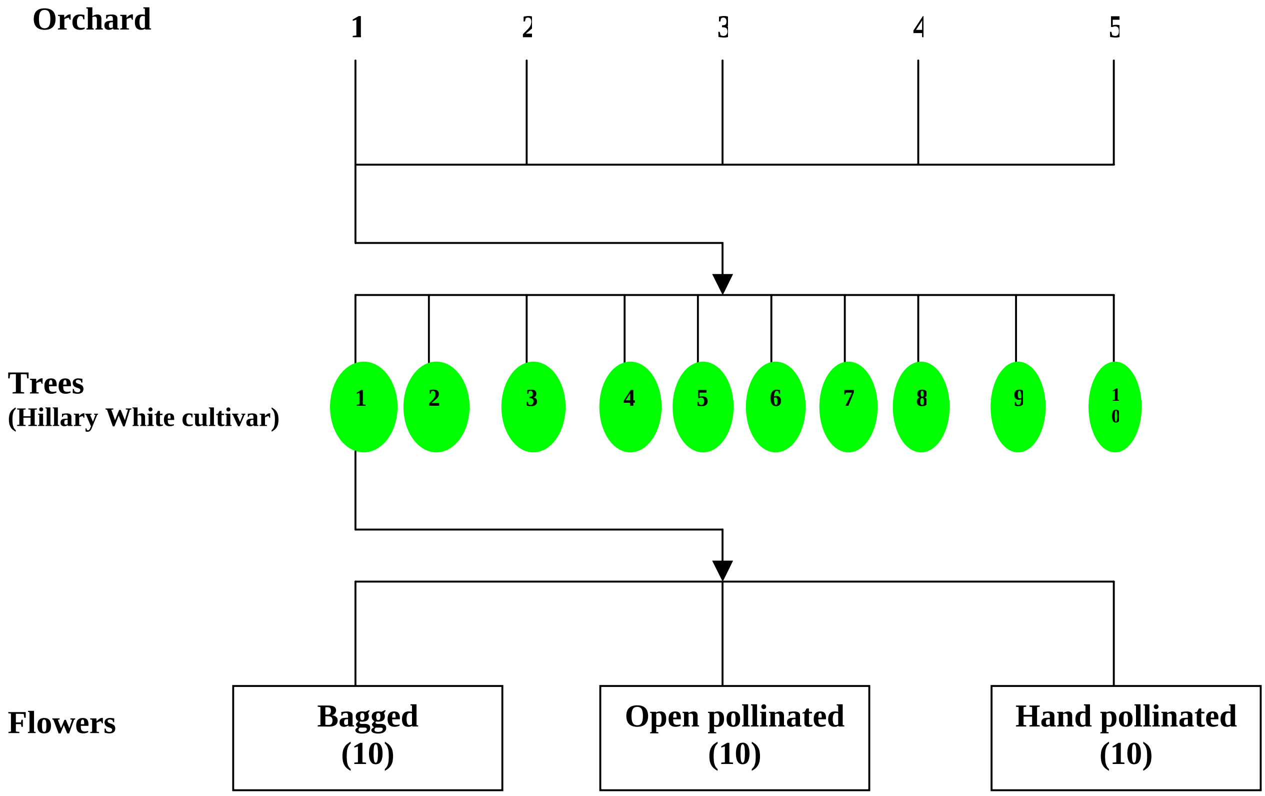

Figure 4.2. Schematic diagram of the experimental design for pollination experiments showing the number of orchards, number of trees within each orchard and the number of flowers per tree used for each of the three pollination treatments.

1) Pollinator exclusion. To determine the proportion of flowers that initiated fruits due to biotic pollination, I had to estimate the percentage fruit set due to self-pollination in the absence of pollinators. To measure the proportion of fruits initiated due to self- pollination I haphazardly selected 10 flowers per tree that were at the bud stage (petals showed no sign of separating from each other). Each flower bud was covered in a fine weave mesh cotton bag (mesh size 0.2mm) that was secured to the pedicle of the flower with a string tie (Figure 4.3). All flower buds were labelled using aluminium tags. The bags prevented visitation from biotic pollinators, thus all fruit initiation in flowers treated this way must be due to self-pollination within the same flower.

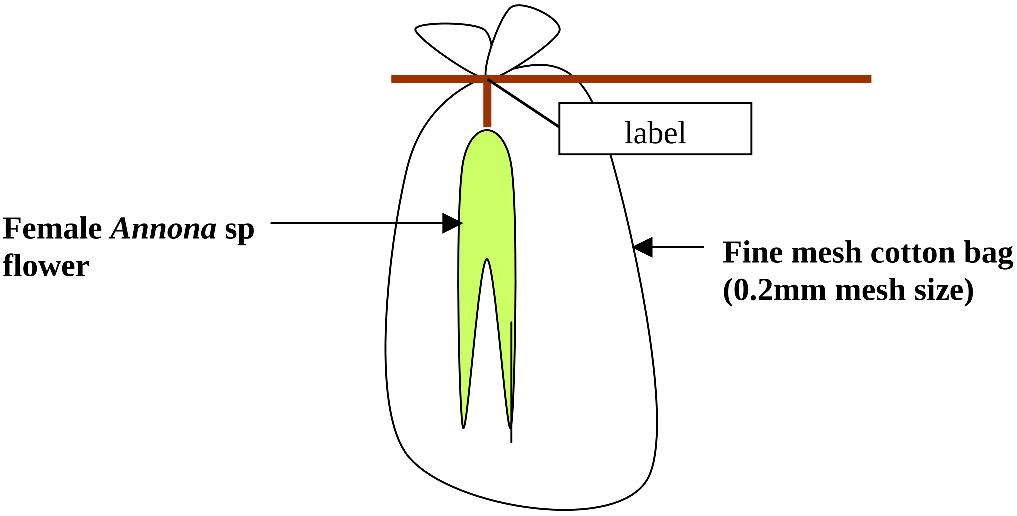

Figure 4.3. Stylised diagram of experimental treatment 1 (pollinator exclusion).

2) Open pollination: Fruit initiation due to both biotic pollination and self pollination was measured in another sample of 10 flowers per tree (Figure 4.2.). This allowed me to compare the proportion of fruits initiated due to biotic agents against the proportion of fruits initiated from self-pollination in the previous treatment. Ten flowers or flower buds were selected haphazardly and labelled using an aluminium tag. All flowers were left untouched (Figure 4.4).

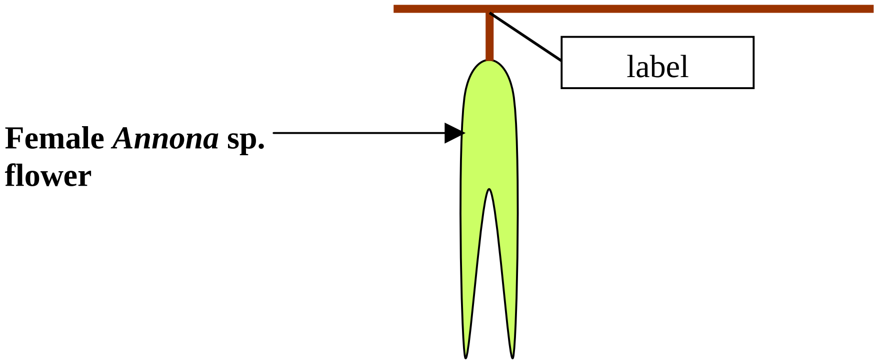

Figure 4.4. Stylised drawing of experimental treatment 2 (open pollination)

3) Hand pollination. Flowers were hand pollinated to determine whether fruit initiation in solely open pollinated flowers was pollen limited. Pollen was collected from flowers of both the Hillary White cultivar and the African Pride cultivar. Pollen was collected by brushing it from the dehiscing anther into a vial using a camel hair painter’s brush. Pollen was collected from the African Pride cultivar at four pm and the Hillary White cultivar at five pm. These are the respective times that the flowers of each custard apple variety transform from the female chamber blossom into a male flower that dehisces and sheds pollen from the anthers.

Pollen of each variety was kept in separate vials and was used to hand-pollinate flowers of separate trees. Five of the ten trees selected for treatments were hand-pollinated using African Pride cultivar pollen and five trees were hand-pollinated using Hillary White cultivar pollen.

Ten Hillary White cultivar flowers per tree in the female reproductive phase were selected haphazardly at five pm to coincide with female receptivity. Flowers can be identified as receptive females because during this phase they produce a strong floral odour, the petals form a floral chamber and the stigmatic surface is moist.

Each of the flowers was augmented with pollen from one of the two varieties. Pollen augmentation was performed by filling a camel hair painter's brush with pollen from either variety. Pollen was obvious on the brush because it is a pale yellow colour and on mass can be seen with the naked eye. The brush was inserted into the female stage flower and twisted three times. The tip of the brush was then smeared through each of the three gaps between the petals. As above, all flowers were labelled with a unique number using aluminium tags.

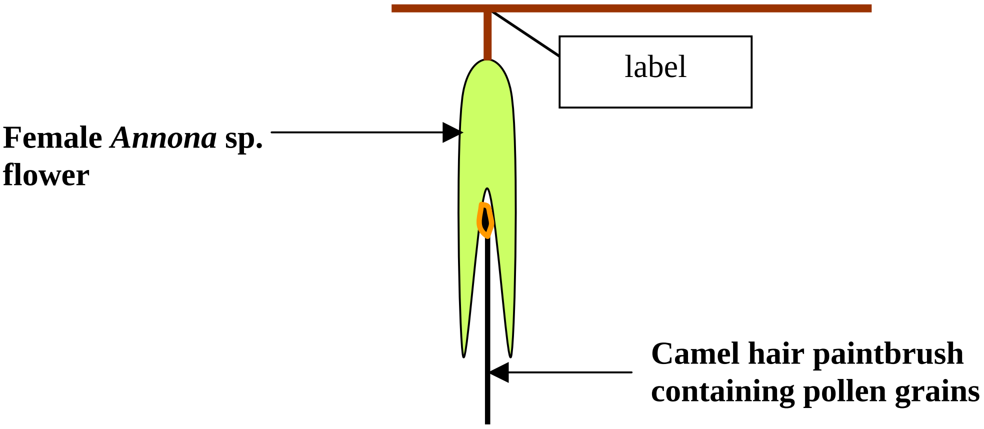

Figure 4.5. Stylised diagram of experimental treatment 3 (hand pollination).

This treatment also estimated whether potential fruit initiation differed between orchard sites due to factors other than the differences in pollination services.

All of the flowers from each of the three treatments were left untouched until flower senescence. After this time, fruit initiation (fruit quantity) was recorded. Fruit initiation can be observed at 10 days after stigma receptivity by the presence or absence of a small aggregate fruit. Mature fruits were collected from the trees at maturity, weighed to the nearest 0.01gram and assessed for symmetry (1= poor symmetry, 5 = good symmetry) (George *et al.,* 1992) (See Appendix 1 for photographic examples of symmetry ranking) (fruit quality). Total number of seeds and the combined mass of seeds and fruit were individually recorded for each fruit.

Analysis

Pollination experiments

I performed a two-way Analysis of Variance with the proportion of flowers initiating a fruit per tree as the dependent variable and orchard (site) and pollination treatment as the two fixed factor independent variables. This allowed me to identify main factor effects and also interaction between site and pollination treatment. Where there were significant differences in the main effects, LSD post-hoc analysis was performed to identify where the differences were apparent.

Orchard isolation from naturally occurring rainforest

While the comparison of means or contingency test was the most appropriate for this data, I was also interested in identifying the nature and magnitude of the relationship between fruit initiation in open pollinated flowers and distance to nearest naturally occurring rainforest. Regression analysis was used to test for a relationship between distance to rainforest habitat and the proportion of flowers initiating fruit. Because I was interested in proportional change in fruit initiation versus the proportional change in distance, I transformed both measures to their natural logarithm and fitted a straight-line relationship between them. In this case, where the estimated slope in log-log space cannot be distinguished from unity (or one) then there is a proportional change. Significant deviations from unity (either positive or negative) indicate exponential increase or decrease respectively. I tested for deviation from unity by determining whether the 95% confidence interval for the estimated regression slope included 1. This transformation had the additional benefit of eliminating the need to fit a curvilinear model, as five data points is too few for such a procedure. Fruit quality measures (mass, symmetry, seed number and seed weight) could not be analysed as a function of distance to naturally occurring rainforest because only five fruits matured from open pollinated flowers. Differences in these parameters between hand pollinated flowers using Hillary White or African Pride sources are considered in Chapter 5.

All statistical tests were performed using SPSS Version 12 (SPSS, Chicago, IL, USA).

Results

Pollination experiments

Both main effects (pollination treatment and orchard site) and the interaction term of the two (pollination treatment x orchard site) indicated significant differences in the proportion of flowers initiating fruit.

Each of the three pollination treatments produced significantly different levels of fruit initiation (ANOVA, $F_{2,135} = 1470.95$, $P = 0.00$) (Figure 4.6). Flowers did not initiate any fruit in the absence of biotic pollinators. Comparatively, an average of 4.48% of flowers that were open to biotic pollinator visits initiated a fruit among the five orchards (Mean proportion ± SE: Briggs Lease (1) 0.11 ± 0.02, Cummings (2) 0.04 ± 0.08, Kilpatrick (3) 0.02 ± 0.04, Lavers (4) 0.02 ± 0.04, Samanes (5) 0.03 ± 0.07).

Flowers that were augmented with pollen from either African Pride or Hillary White cultivar trees produced substantially more fruit than either of the other two treatments. An average of 90.52% of flowers within the hand pollination treatment initiated a fruit (Mean proportion ± SE: Briggs Lease (1) 0.83 ± 0.18, Cummings (2) 0.99 ± 0.03, Kilpatrick (3) 0.76 ± 0.25, Lavers (4) 0.95 ± 0.11, Samanes (5) 0.98 ± 0.42) (Figure 4.6).

Kilpatrick orchard differed overall from the other four orchards (ANOVA, $F_{4,135} = 3.46$, $P = 0.01$). This effect appears to be produced because fewer fruits are initiated in flowers that were augmented with pollen in the hand pollination treatment compared with other orchards and does not appear to differ in fruit initiation for the other two pollination treatments (Figure 4.6).

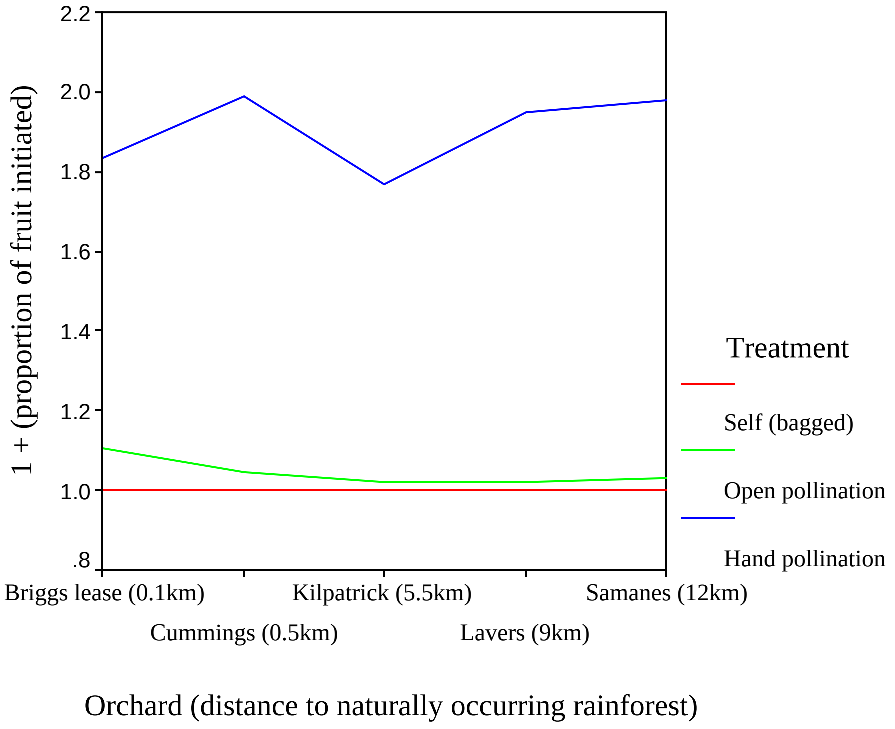

Figure 4.6 Proportion of custard apple (*Annona squamosa* x *Annona cherimola*) flowers initiating a fruit in each of three pollination treatments: bagged flowers, open pollinated flowers and hand pollinated flowers.

Within site differences

Although the power of this statistical test is not ideal, having only five points within the model, I determined that it is a worthwhile exercise to plot this data to attempt to find a trend, at least that may indicate differences in open pollination across orchards. I also propose that determining this relationship is necessary because of the significant relationship I found between floral visitor abundance and species richness and orchard distance from naturally occurring rainforest (or at least orchard location in the landscape) where a sufficient number of data points were used. Because of the known dependence of custard apple production on biotic pollination I expected a similarity between the relationships (orchard location x floral visitors; orchard location x fruit initiation) in spite of the small data set; and this seems to be the case.

Flowers only initiated a fruit when visited by biotic pollinators. When orchards were treated independently, the orchard located nearest to rainforest habitat produced significantly more fruits than any other orchard suggesting that there are greater pollination services in this orchard (Figure 4.7). An average of 10.86% of flowers initiated fruit in Briggs orchard (100 metres from rainforest habitat). Comparatively, no more than 4.4% of flowers initiated a fruit in the other four orchards located 500 metres or more from rainforest habitat.

Taking the natural logarithm of both variables, the variation in fruit initiation due to biotic pollination among the five orchards can be partially explained by the proximity of an orchard to rainforest habitat ($R^2 = 0.21$, $F_{1,48} = 12.90$, $P = 0.001$). The equation for the relationship is lny (distance) = ln0.52 - 0.015 lnx (proportion of flowers initiating a fruit).

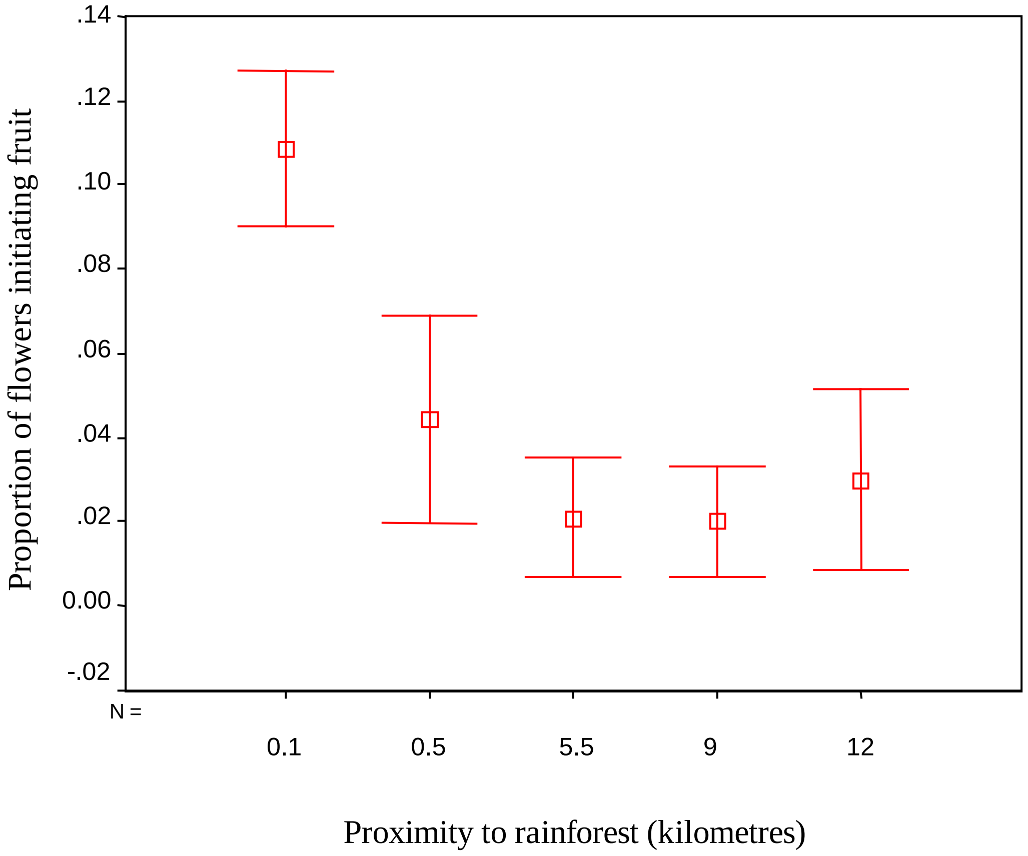

Figure 4.7. Proportion of open pollinated flowers (10 flowers x 10 trees) initiating a fruit at five orchards located at increasing isolation from naturally occurring rainforest habitat.

Because most flowers initiated a fruit in hand pollination treatment and their was no obvious relationship with distance (Figure 4.8) there was no relationship between the proximity of an orchard to naturally occurring rainforest and the ability of a flower to initiate a fruit when sufficient pollen was supplemented to female flowers through the hand pollination treatment ($R^2 = 0.002$, $F_{1,48} = 0.104$, $P = 0.749$).

For example, hand-pollination treatment in Kilpatrick orchard located at 5.5 km from naturally occurring rainforest produced significantly fewer fruits than all orchards except Briggs Lease that is located 100 m from naturally occurring rainforest.

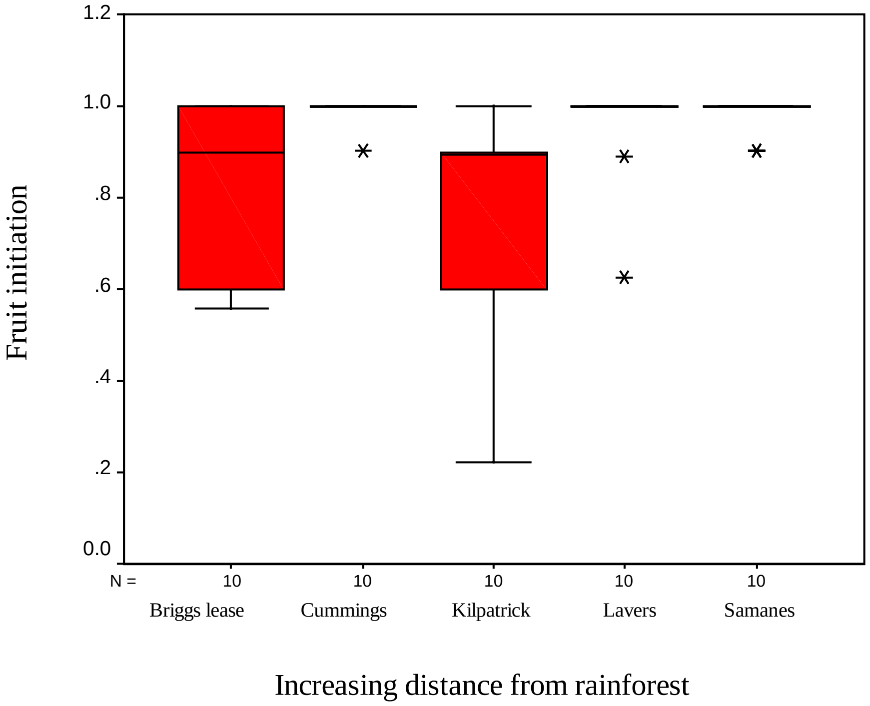

Figure 4.8. Proportion of custard apple (Annona squamosa x Annona cherimola) flowers initiating a fruit after hand pollination in five orchards located at increasing distance from rainforest habitat.

Discussion

In this chapter I have shown that custard apples (*Annona squamosa* x *Annona cherimola*) on the Atherton Tablelands, north Queensland, do not produce any fruit in the absence of biotic pollinators. Therefore, all fruit production in open-pollinated flowers can be attributed to biotic pollinator agents in these orchards. Complete failure of fruit set in flowers where pollinators were excluded is not common in other custard apple growing regions, however, the percentage attributable to self-fertilisation has not exceeded 1.5 % in any reported cases (Thakur & Singh 1964, George *et al* 1989), indicating that self-fertilisation in the absence of pollinators, while possible, is rare in this cultivar.

Fruit initiation due to biotic pollinators (open pollinated flowers) was low compared to the number of flowers available for fertilisation. The proportion of flowers producing a fruit ranged between 2.0% and 11.0%. This low fruit initiation could be attributed to pollen limitation because receptive female flowers that were supplemented with pollen initiated significantly more fruit than those that were solely open pollinated (Figure 4.6). In fact fruit initiation could be increased by more than 50% if flowers were supplemented with pollen. This indicates that pollinators are not transferring sufficient pollen to receptive stigmas to maximise fruit set or alternatively the quality of pollen being transferred is poor, resulting in fruit abortion. This is not uncommon in commercial custard apple orchards. In other regions such as northern India, Israel, southeast Queensland, Australia and the United States fruit set levels are typically low (5% - 25%) (Thakur & Singh 1964, Gazit *et al* 1982, George & Nissen 1988 and George *et al* 1989, Schroeder 1995). Other authors have identified pollen limitation, as well as physiological stress, as the primary factors producing low yields in this crop (Shroeder 1995, Peña *et al* 1999). Because of low productivity in custard apple crops, many researchers have attempted to increase pollinator populations in orchards through providing breeding sites (rotting fruit) or pheromone attractants for the primary pollinator species (usually Nitidulid beetles), with some success (Peña *et al* 1999), however, few studies have attempted to elucidate which larger scale factors initially influence pollinator community composition and abundance in the landscape.

Studies of other crop species such as watermelon, macadamia nut, radishes and mustard, identify landscape structure (crop isolation and proportion of native habitat in a landscape) as a strong determinant of the abundance and species richness of a pollinator community (Heard & Exley 1994; Steffan- Dewenter & Tscharntke 1999, Kremen *et al* 2002, Steffan-Dewenter *et al* 2002, De Marco Jr P & F.M. Coelho 2004). The contribution of native species to fruit production in these crops appears to be quite high, so that suppressed fruit productivity results from the absence of some or all of these species.

Landscape structure directly influences productivity in these crops, and this also appears to be the case for custard apples.

Fruit initiation was highest in Briggs Lease orchard. Fruit initiation in this orchard was significantly higher than all the other orchards and these did not differ significantly from each other. This orchard is located at closest proximity to rainforest habitat (100 metres), and I had wanted to isolate this effect as a factor determining both floral visitor abundance and species richness and fruit production in custard apple orchards, however, because of the lack of replication in the variable distance from naturally occurring rainforest I can only determine that fruit initiation (and floral visitor abundance and species richness; see Chapter 3) was influenced by position of the orchard in the landscape, be it because of distance to naturally occurring rainforest, rainfall or some other factor associated with the landscape gradient. There is a rainfall gradient associated with the gradient of orchard location relative to naturally occurring rainforest and these two are strongly correlated across this landscape, however, in this study and Blanche and Cunningham (2005) have elucidated that in the same landscape, custard apple floral visitor abundance and species richness were more strongly correlated with distance to naturally occurring rainforest indicating that it is the more determinant factor.

There was no relationship between distance to rainforest and the ability of a flower to initiate a fruit when pollen was added to receptive stigmas, in fact fewer fruits were initiated from flowers in the orchard closest to rainforest habitat than three of the other orchards. This suggests that all flowers in the orchards located at distance from rainforest are able to initiate fruits but biotic pollinators are transferring more pollen to flowers in Briggs Lease orchard. This could be because pollinator species in this orchard are more effective at transferring pollen or because there are more of them. Beetle pollinators (Family Nitidulidae) in other custard apple growing areas such as Israel and California have been found to be equivalent in their ability to transfer pollen (Gazit *et al* 1982, Nagel *et al* 1989). It is difficult to suggest that this is the case on the Atherton Tablelands, however, because a large proportion of the arthropod floral visitors in Atherton Tablelands orchards are new records for custard apple pollination. Therefore, it is not known whether these particular species differ in their effectiveness as pollinators.

Both arthropod abundance and species richness was significantly higher in the orchard closest to rainforest habitat (see Chapter 3). These two factors cannot be separated in this study. Regardless of which factor is more important, it is clear that landscape structure is an equally important determinant of a pollinator community and resultant crop productivity in a commercial Australian tropical crop, as it has been demonstrated to be in other crops in temperate Australia and other parts of the world.

Chapter 5

Supplementary pollination in the production of custard apple (Annona sp.) – effect of pollen source

Abstract

Low natural pollination rates produce few, poorly formed, fruits in commercial orchards of the custard apple (*Annona squamosa* x *A. cherimola*) cultivar 'Hillary White' on the Atherton Tablelands, North Queensland, Australia. To overcome this limitation, conventional practice is to hand-pollinate with pollen from either cu. 'Hillary White' or cu. 'African Pride'. Supplementary pollination, using either pollen type, significantly increased overall fruit production and fruit quality above natural levels. However, pollen sourced from cu. 'African Pride' trees produced significantly larger and more symmetrical fruits than pollen from cu. 'Hillary White' itself. Increased quality was not at the expense of quantity. There was no difference in mean fruit yield between flowers supplemented with pollen from either variety. These results indicate that using cu. 'African Pride' pollen should result in greater economic returns for growers through the production of a higher proportion of 'best' quality fruits.

Introduction

Fruit production rates and resultant fruit quality are principal determinants of economic returns in commercial fruit crops. The successful transition from flower to fruit is contingent on a number of factors operating within a single flowering period, including pollen availability, pollen quality, resource availability and predation and disease (Ayre & Whelan 1989). These factors limit fruit production such that total fruit number is generally less than the maximum number of flowers available for fertilization. Two important determinants of successful fruit production are pollen quantity (number of grains reaching a stigma) and pollen quality (pollen source). Both have been shown to influence productivity and quality of fruits produced in wild plant species and commercial fruit cultivars (Lee 1988 (and references therein), Ayre & Whelan 1989, Wallace and Lee 1999, Voyiatzis & Paraskevopoulou-Paroussi 2002).

Pollen quantity limits fruit production because the number of pollen grains reaching receptive stigmatic surfaces determines the upper limit to the quantity of seeds that are fertilized within a flower; seed number is positively related to the probability of fruit initiation and fruit maturation (Lee 1988). When pollen quantity limits fruit production, pollen additions to receptive flowers increases the proportion of flowers producing fruit. Experimental evidence for this effect is well established in: *Tipularia discolor, Epidendrum ciliare, Dendrobium monophyllum* (Orchidaceae), *Telopea speciosissima* (Proteaceae), *Blandfordia nobilis* (Liliaceae) and *Annona* sp. (Annonaceae) (Snow &

Whigham 1989, Ackerman & Montalvo 1990, Bartareau 1995, Schroeder 1995, Goldingay 1998).

Pollen quality influences resultant fruit production because pollen varies in its viability and compatibility with the maternal plant. Often self pollen or "unfavoured" pollen donors germinate fewer pollen grains on receptive stigmatic surfaces of maternal plants and grow fewer pollen tubes to fertilise ovules that would result in seeds (Lee 1988). Alternatively, seeds from unfavourable parentage may produce unfavourable gene combinations resulting in homozygous recessive harmful alleles that result in seed abortion (Lee 1988). Seed number is positively related to fruit growth because seeds produce phytohormones (auxin and giberellin) that stimulate fruit development and fruits containing fewer seeds are more likely to abort (Lee 1988).

Pollen quantity and quality may also interact to determine final fruit quality and these measures may not be independent. Interactions arise because some economically important fruit quality parameters (size and shape) reflect the number of seeds matured within a fruit (McGregor 1976). For example, fruit size is positively related to seed number because auxin (a phytohormone produced by developing seeds) stimulates the development of adjoining tissue, increasing fruit mass (McGregor 1976).

The number and distribution of developing seeds within a flower (or flowers) may also influence fruit shape. For example, aggregate fruits are a cluster of 'druplets' that each develops from a successfully pollinated pistil within a single flower (Raven *et al* 1999).

The number and distribution of successfully pollinated individual pistils determines the distribution of developing seed and associated fruit mass and ultimately defines the 'symmetry' of the aggregate fruit.

Custard apples (*Annona cherimola* x *Annona squamosa*: Annonaceae*)* are aggregate fruits. Flowers are specialised into a floral chamber with three fleshy white/green petals enclosing multiple male and female sex organs. Multiple pistils are fertilized during pollination and each pistil forms a 'druplet', which fuse to form a single aggregate fruit. Commercially grown varieties of custard apples typically produce few poor quality fruit from natural pollination and low productivity has been attributed to pollen limitation and/or physiological stress (Schroeder 1995, Pẽna *et al* 1999). In southeast Queensland, natural pollination rates range from 5.7% to 25% (George *et al* 1989, George *et al* 1992) and in this region growers hand-pollinate flowers to increase productivity to economically viable levels. Natural pollination rates in commercial custard apple crops in the tropical north Queensland are also low (see Chapter 4). In this region, hand-pollination is also used to increase fruit yield, however, fruit initiation and development is variable between hand-pollinated flowers both among and within trees indicating that mechanisms other than pollen limitation alone limits fruit production (Briggs pers. comm.). On the Atherton Tablelands all fruit bearing trees are the 'Hillary White' cultivar. Conventional practice is to use both Hillary White and African Pride varieties as pollen sources for hand-pollination, therefore, one potential mechanism limiting fruit production may be pollen quality (pollen source) rather than pollen quantity.

Surprisingly, there has been no investigation of the effects of pollen source on either fruit production or resultant fruit quality to date.

In this study I test whether pollen source (African Pride or Hillary White) used in hand pollination of Hillary White cultivar custard apple influences fruit initiation (quantity) and resultant fruit quality (mass and symmetry).

Methods

The experiment was carried out during October 2002 at five commercial custard apple orchards on the Atherton Tablelands, Queensland, Australia (see Chapter 2, Section 2.2: *Orchard sites*). In each orchard, ten mature 'Hillary White' cultivar trees were selected from a pure block planting and used for pollination experiments.

In each orchard, five of the ten trees were assigned to one of two treatments to test for differences in fruit production due to pollen source used in hand pollinations, treatments were: 1. Hand-pollination using 'Hillary White' cultivars pollen and 2. Hand-pollination using 'African Pride' cultivars pollen. In each of the ten trees per orchard, ten receptive female stage flowers were used for hand pollination treatments (Figure 5.1).

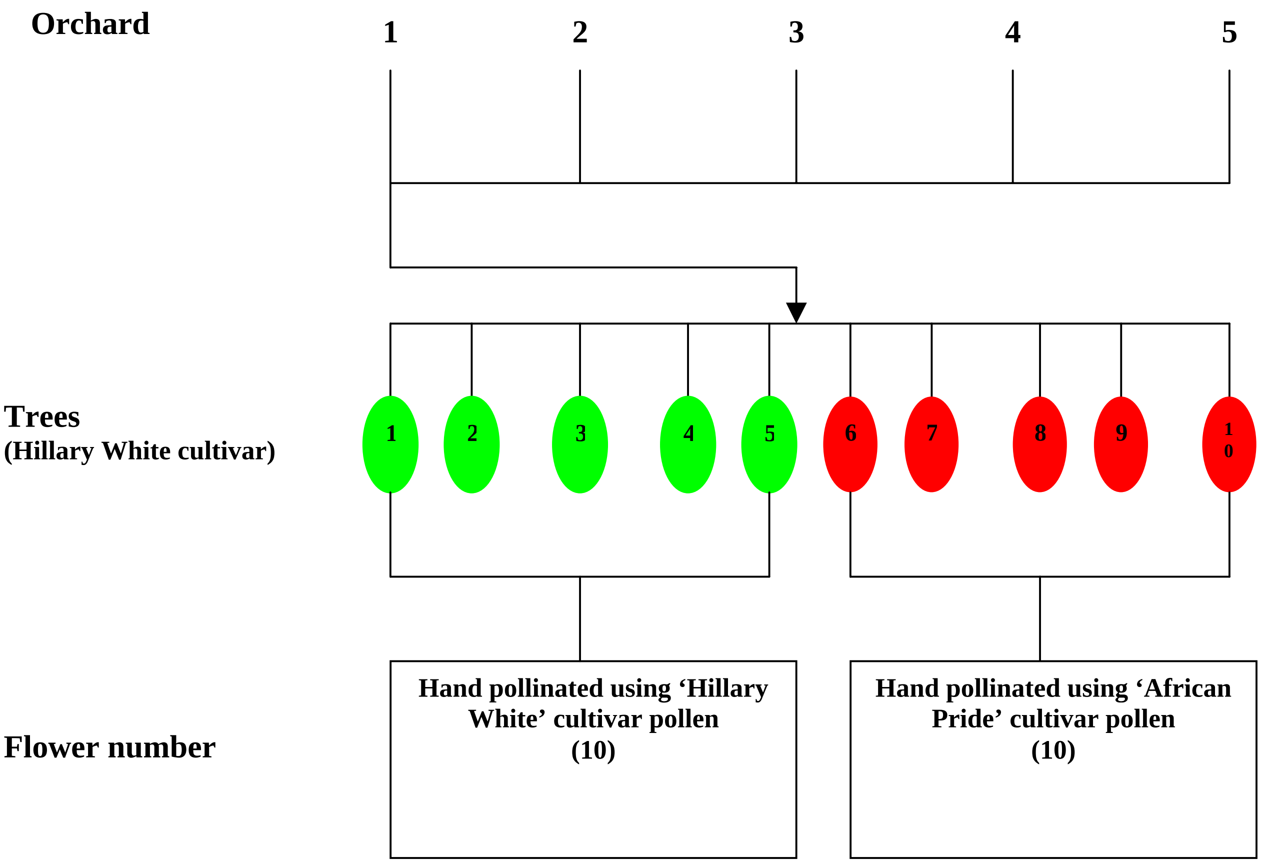

Figure 5.1. Schematic diagram of experimental design for testing differences in fruit production between flowers hand pollinated using 'African Pride' cultivar pollen and flowers hand pollinated using 'Hillary White' cultivar pollen. Orchards and trees are the same used in experimental treatments in Chapter 4. Five trees in each orchard were assigned to one of the two treatments. Ten flowers from each tree in the receptive female stage were supplemented with pollen from one of the two varieties.

To measure fruit production due to hand pollination (and pollen source) treatment, ten receptive female flowers per tree were hand-pollinated using pollen sourced from one of the two custard apple cultivars. Receptive female flowers were those that had moist stigmatic surfaces and produced a strong floral odour. Pollen was collected from the African Pride cultivar at four pm and the Hillary White cultivar at five pm. These are the respective times that the flowers of each custard apple variety transform from the female chamber blossom into a male flower that dehisces and sheds pollen from the anthers.

Pollen was collected by brushing it from the dehiscing anther into a vial using a camel hair painter's brush. Pollen of each variety was kept in separate vials and used to hand-pollinate flowers of separate trees. Each flower was augmented with pollen from one of the two varieties immediately after pollen collection. This was done by inserting the brush into the female stage flower and twisting three times. The tip of the brush was then smeared through each of the three gaps between the petals. The flower was labeled using an aluminium tag.

All flowers from each treatment were left untouched until flower senescence at which time fruit initiation (fruit quantity) was recorded. Fruit initiation can be observed 10 days after stigma receptivity as the presence of a small aggregate fruit. Fruits were allowed to grow to maturity and then collected directly from trees, weighed (to the nearest 0.01gram) and assigned a symmetry value (1 = poor symmetry, 5 = good symmetry)

Symmetry categories were determined by the proportion of fruit that was malformed (undeveloped) (that is, category 1 fruit contained equal to or greater than 4/5 of the fruit undeveloped, category 2 = 3/5, category 3 = 2/5, category 4 = 1/5 and category five had no area of the fruit malformed) (Figure5.2).

Category 1

Category 2

Category 3

Category 4

Category 5

Figure 5.2. Examples of custard apple fruit from each symmetry category.

Finally, samples of mature fruits from both treatments were dissected to count the number of seeds (total number = 134). This was done to assess whether there was a relationship between both seed number and resultant fruit mass and seed number and resultant fruit symmetry indicating that pollination success in custard apples directly influences fruit quality.

Analysis

Fruit productivity (proportion of flowers that initiated a fruit) was compared between pollen source treatments (African Pride pollen vs. Hillary White pollen) using a non-parametric Mann-Whitney *U*-test, because data violated the assumption of homoscedasticity for parametric tests. One-way ANOVA was used to compare mean fruit mass between flowers hand pollinated using Hillary White pollen and flowers hand pollinated using African Pride pollen. Seed number per fruit was compared between treatments of African Pride and Hillary White pollen using one-way ANOVA. Fruit quality was compared between pollen source treatments using chi-square homogeneity. Regression analysis was used to test for a relationship between seed number and fruit mass and non-parametric Spearmans rank correlation was used to examine the relationship between seed number and fruit symmetry. All tests were performed using SPSS Ver.12 (SPSS, Chicago, IL, USA).

Results

In hand pollination treatments, pollen source did not affect the proportion of flowers initiating fruit. Flowers pollinated using African Pride cultivar pollen (0.854 ± 0.043 SE) initiated the same number of fruits as flowers pollinated using Hillary White cultivar pollen (0.957 ± 0.043 SE; Mann-Whitney *U*-test = 244.50, *P* = 0.130) (Figure 5.3). Fruit quality parameters varied in response to pollen source. Fruit quality was significantly different between fruits hand pollinated using African Pride cultivar pollen and Hillary White cultivar pollen. Hand pollination using African Pride pollen produced larger fruit than Hillary White cultivar pollen ($F_{2,\,203} = 7.073$, $P = 0.001$) (Figure 5.4).

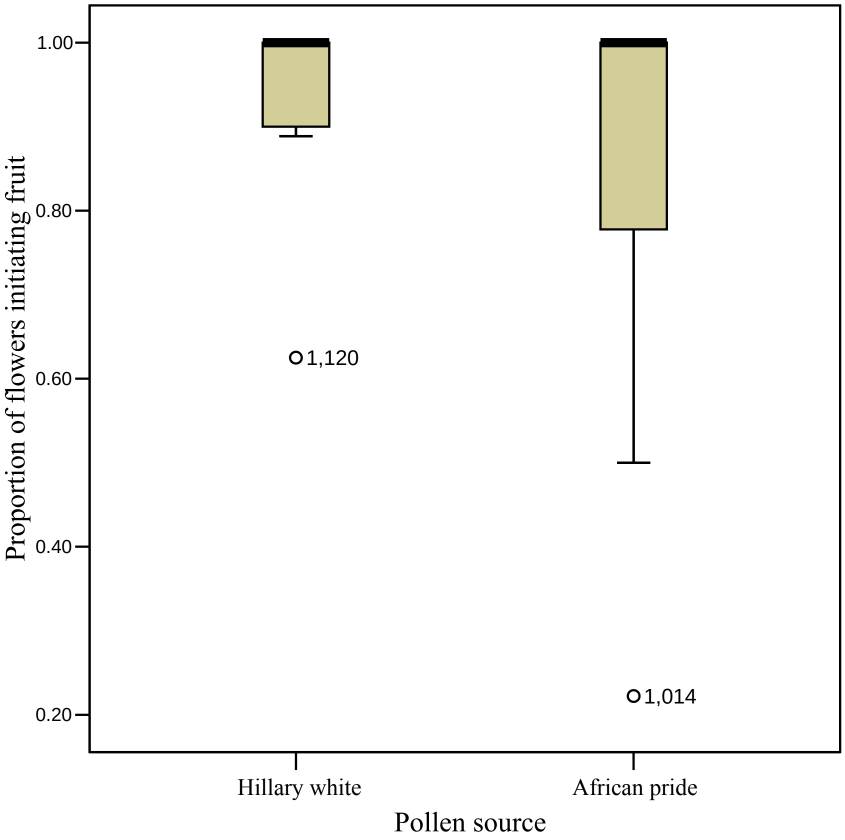

Figure 5.3. The proportion of flowers that initiated a fruit in flowers hand pollinated using either Hillary White cultivar pollen or African Pride cultivar pollen.

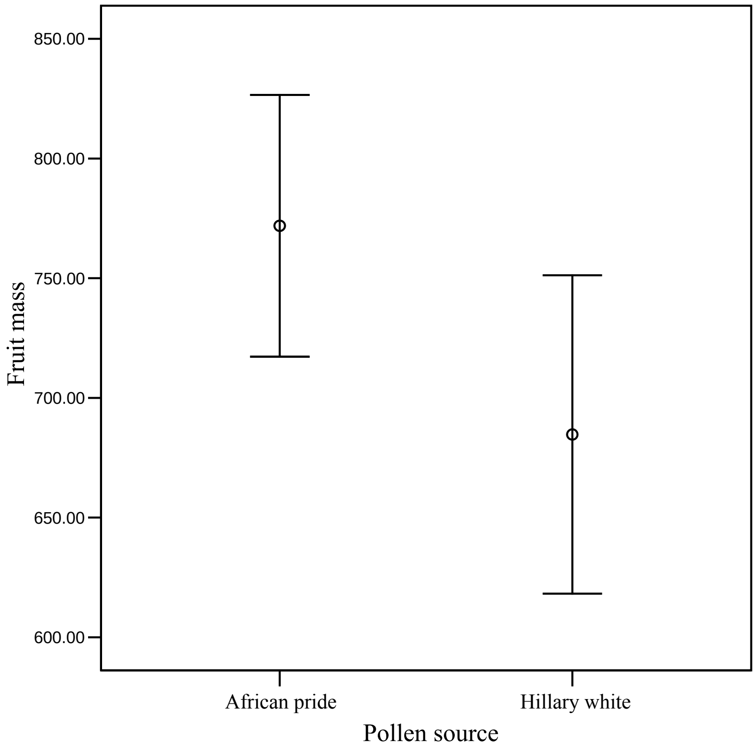

Figure 5.4. Custard apple fruit mass of mature fruits resulting from flowers hand pollinated using pollen sourced from African Pride cultivar or Hillary White cultivar trees.

The difference in mass between African Pride and Hillary White pollination treatments reflected the number of successfully provisioned seeds. The number of seeds per fruit was significantly greater in fruits produced from African Pride pollen (26.15 ± 1.78SE) compared with those produced from Hillary White pollen (18.51 ± 1.96SE; $F_{1,199} = 7.77$, $P = 0.006$) and fruit mass was significantly positively related to the number of seeds produced within a fruit ($R^2 = 28.8$, $F_{1,132} = 53.49$, $P = 0.00$) (Figure 5.4).

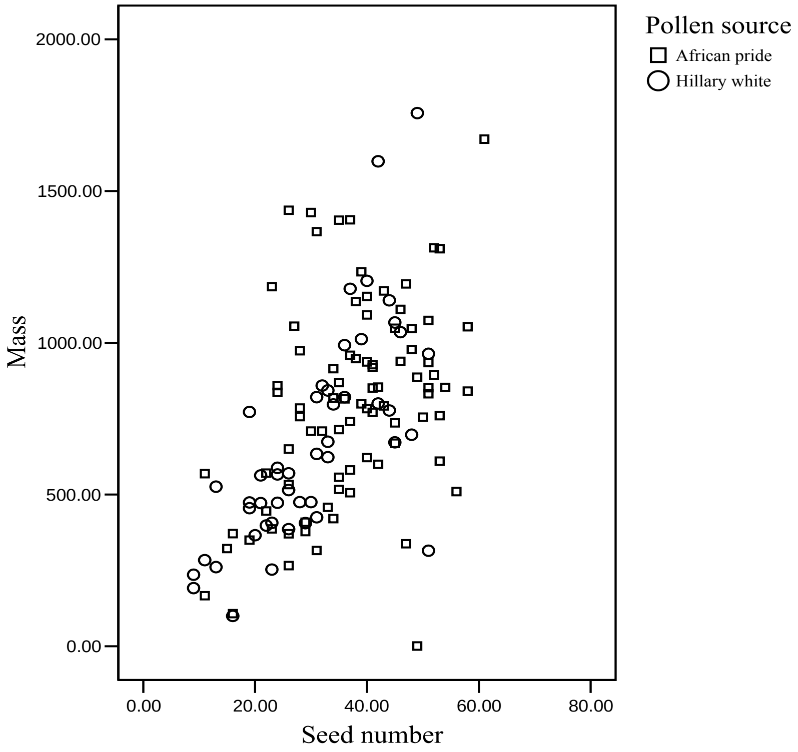

Figure 5.5. Relationship between fruit mass and seed number per fruit for fruit resulting from hand pollinations using pollen sourced from African Pride and Hillary White cultivar trees.

Pollen source also influenced the shape (symmetry) of fruit. African Pride pollen produced a greater than expected number of fruit with perfect symmetry (Category 5) compared to Hillary White pollen. Hillary White pollen produced a greater than expected number of fruit with almost perfect symmetry (Category 4) however, there was significantly more fruits with poor symmetry (Categories 1-3) resulting from Hillary White pollen than from African Pride pollen (Categories 1-3) ($\chi^2 = 16.954$, df = 5, $P = 0.005$) (Fig. 5.6). Seed number was positively correlated with fruit symmetry (Spearmans Rank Correlation = 0.612, $P = 0.00$, n = 135) (Figure 5.7) indicating that pollination success directly translates into fruit mass and fruit symmetry.

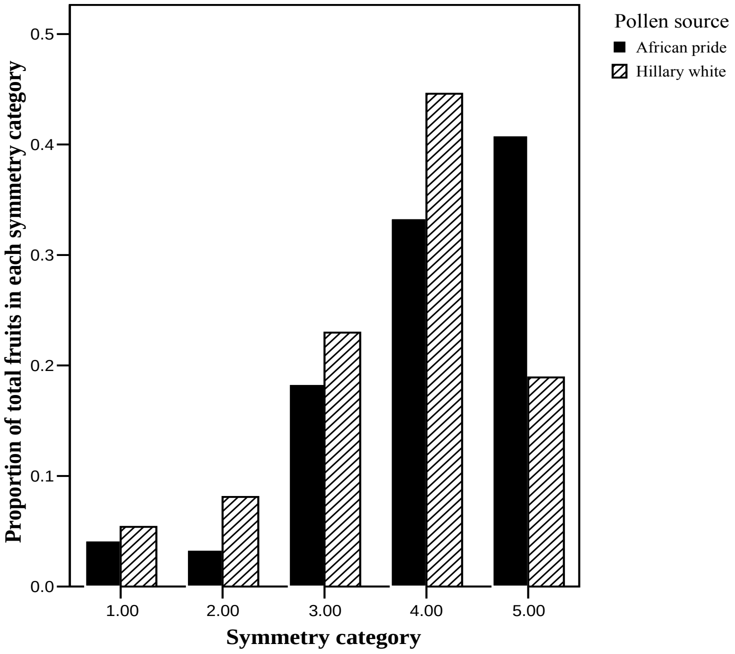

Figure 5.6. Relative proportion of fruit in each symmetry class (1 = poor symmetry, 5 = good symmetry: see methods for photographs of representative fruit from each symmetry class) for fruits produced from flowers hand pollinated using pollen sourced from either African Pride and Hillary White cultivar custard apple trees.

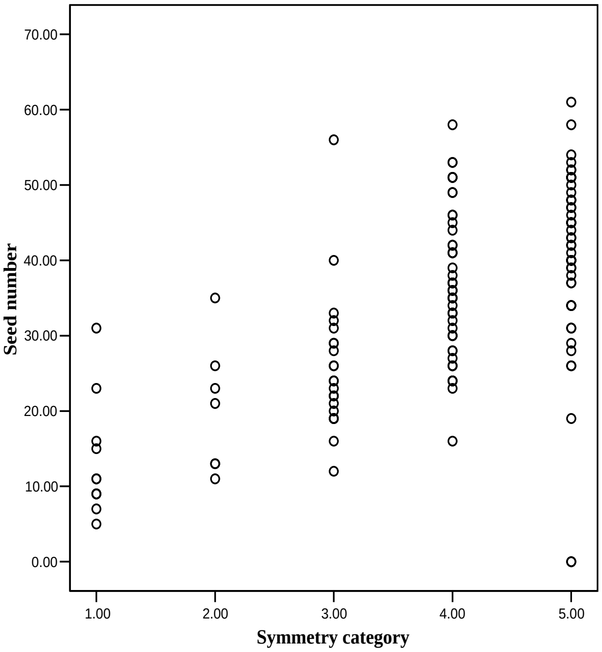

Figure 5.7. Correlation between total seed number per mature fruit and the symmetry category (shape).

Discussion

Custard apple production in north Queensland orchards is limited by natural rates of pollination. We showed that average natural pollination rates in orchards did not exceed 22% per tree and in some orchards was well below this value (Chapter 4). These findings are similar to rates measured in other custard apple growing regions e.g. Florida, south-east Queensland, Australia and New Delhi (Thakur & Singh 1964, George & Nissen 1988, Nagel *et al* 1989, George *et al* 1992, Peña *et al* 1999). Custard apples are predominantly beetle pollinated and the low pollination rate has been attributed to low abundance of beetles visiting flowers. Higher 'natural' pollination rates (50-66% fruit set) have been achieved in Florida and Israel when pollinator abundance is artificially increased by caging beetles around flowers or attracting greater numbers to orchards with pheromone baits (Gazit *et al* 1982 and Nadel & Peña 1994). Large scale mechanisms influencing pollinator abundances in orchards are poorly understood, although landscape structure (isolation from native habitat) appears to determine the diversity and abundance of floral visitors in custard apple orchards (Chapter 3) as it has been shown to do in other crops such as mustard and radishes in Germany (Steffan- Dewenter & Tscharntke 1999) and highland coffee in Central Sulawesi (Klein, Steffan-Dewenter & Tscharntke 2003). In the absence of implementing any mechanism likely to increase natural pollinator attendance to orchards, hand pollination is required to produce an economically viable crop.

On the Atherton Tablelands, hand pollinating custard apple flowers resulted in a substantial (and significant) improvement in fruit initiation and resultant fruit quality.

Average fruit production ranged between 76.8% and 99% and there were no differences in the proportion of flowers initiating a fruit when hand pollinated with either Hillary White or African Pride variety pollen. Pollen limitation, therefore, appears to be the primary mechanism limiting initial fruit production.

Post fruit initiation, fruit quantity and fruit quality were influenced by pollination treatments and can be related to successful seed maturation. Fruits matured from flowers hand pollinated using African Pride cultivar pollen were larger and more symmetrical than fruit matured from flowers pollinated using Hillary White cultivar pollen. This result has been found in other commercial crop species; pollen source has been shown to determine fruit quality (fruit weight and sugar content) in mandarin cultivars. Fruit weight of ‘Imperial’ mandarins is significantly higher when pollen is sourced from ‘Ellendale’, ‘Murcott’ and ‘Ellenor’ variety trees (92-103g) compared with self ‘Imperial’ pollen (33-55g). Similarly, sugar content in ‘Imperial’ mandarins is significantly higher when pollen is sourced from ‘Ellenor’ (8.71%) and ‘Murcott’ (8.68%) variety trees than when fruit is pollinated using self-pollen (7.12-7.78%) (Wallace & Lee 1999). Both Wallace & Lee (1999) and the evidence presented here suggest that fruit quality is increased if hand pollination treatments use cross-cultivar pollen than within cultivar pollen.

Differences in fruit mass and fruit symmetry between hand pollination treatments largely reflected differences in the total number of successful pollination events (seed production) associated with pollen type. The total number of seeds per fruit was significantly higher in fruit pollinated with African Pride pollen compared with those pollinated with Hillary White pollen and total seed number was positively associated with large fruit size and better fruit symmetry. Consequently, in relation to both mass and symmetry, ensuring pollination success in the largest number of ovules within individual flowers should return highest quality and largest quantity of fruit in this crop. Differences in successful seed production may be due to either pre or post zygotic failure. Pre-zygotic seed failure can result from inferior pollen aborting at the stigmatic surface or during pollen tube growth that reduce the total number of fertilized seeds within a developing fruit. Post-zygotic mechanisms for lower seed production have been attributed to seed abortion or reduced competitive ability between seeds that are formed from ‘unfavourable’ gene combinations that can contain deleterious alleles (Lee 1988). There is evidence that seed number is lower in fruits resulting from self pollinated flowers or in commercial plants species that are fertilized using pollen from the same cultivar indicating that there is a genetic basis for seed abortion (Lee 1988).

Fruit quality (size and shape) is an important determinant of the market value of custard apple fruit. Larger, more symmetrical fruit gain higher prices than small, poorly formed ones. Understanding the potential influences that contribute to fruit size and shape is very important if commercial producers wish to realize maximum returns. Our results show significant differences in fruit size and symmetry associated with pollen source and hand pollination using cross-cultivar pollen produced larger and more symmetrical fruit. This is contrary to conventional hand pollination practice where pollen is haphazardly sourced from either African Pride or Hillary White cultivar trees for hand pollinating flowers.

Given that improvements in fruit quality parameters were not accompanied by a decrease in fruit number, the use of pollen sourced from African Pride should provide a better economic outcome for custard apple growers.

Chapter 6

Synthesis

Biotic pollination represents an essential ecosystem service provided by native habitats and their component species to crops. Pollination services are proposed to be declining in both native ecosystems and agro-ecosystems in various parts of the world (Buchmann & Nabhan 1996). This decline is demonstrated through empirical evidence of both pollinating species declines and increasing pollination limitation (lower fruit and seed set) in native and crop plant species (Buchmann & Nabhan 1996, 1999, Allen-Wardell *et al* 1998).

Habitat loss is one factor suggested to be responsible for declines in pollination services. As the area of land under cultivation increases (crop expansion) across a landscape, the area of remnant native habitat necessarily decreases. This results in a landscape containing less area of native habitat relative to crop area and crop areas that are increasingly isolated from remnant native habitats. Reduction in the area of native habitat reduces the number of species and individuals contained within that habitat resulting in fewer pollinators servicing larger areas of crops (Kearns et al 1998, Kevan & Phillips 2001). Isolation of crops from native habitats has been found to reduce the species richness and abundance of native pollinator species servicing crop plants resulting in declines in fruit and seed production (Steffan- Dewenter & Tscharntke 1999, Steffan-Dewenter *et al* 2002, Ricketts 2004).

The native ecosystems and inherent species responsible for providing ecosystem services such as pollination to agriculture are largely unknown. In this study I have surveyed the suite of potential pollinators that service a tropical horticultural crop (*Annona squamosa* x *A. cherimola*: custard apple cu ‘Hillary White’) grown commercially on the Atherton Tablelands, north Queensland. I found 19 species of floral visitors in total, (18 beetles and 1 thrip), 3 of these species are exotic, 3 of these species are known natives and 13 species are of unknown origin and have the potential to be natives deriving from naturally occurring rainforest. Because of the scarcity of taxonomic work carried out on these groups, I was unable to identify many of the samples to species; however, all but 3 species (*Carpophilus* spp.) are potential new records for custard apple pollinators.

Landscape structure, or the position of the orchard in the landscape across a north to south gradient that includes parameter gradients such as increasing distance from naturally occurring rainforest and decreasing rainfall, was negatively related to species richness and abundance of floral visitors (both when analyses were performed for all species and all species excluding known ‘exotics’) in the custard apple orchards. The species richness and abundance of the floral visitor assemblage declined exponentially with the gradient (distance from naturally occurring rainforest and rainfall) indicating that at least a proportion of the species could be rainforest derived and are limited by either rainfall or some mechanism to disperse to orchards beyond approximately 400 metres from naturally occurring rainforest (Figure 6.2).

Custard apples grown on the Atherton Tablelands do not self-pollinate; flowers do not produce fruit in the absence of biotic pollinators. Furthermore, managed honeybees are not effective pollinators at all and may have a negative effect on fruit production by robbing male flowers of pollen (pers. obs). This means that unlike other crops that rely on managed honeybees for pollination, for custard apples, honeybees cannot replace a depauperate pollinator fauna in the landscape.

The negative relationship between floral visitor species richness and abundance and the landscape gradients was associated with a negative relationship between fruit initiation and the position of the orchard across the landscape (distance to naturally occurring rainforest) indicating that the decline in arthropod visitors to custard apple flowers results in poorer fruit initiation in these orchards. Fruit initiation declined significantly in orchards located greater than 500 metres from naturally occurring rainforest and variability in fruit production increased substantially beyond 100metres from naturally occurring rainforest (Figure 6.2).

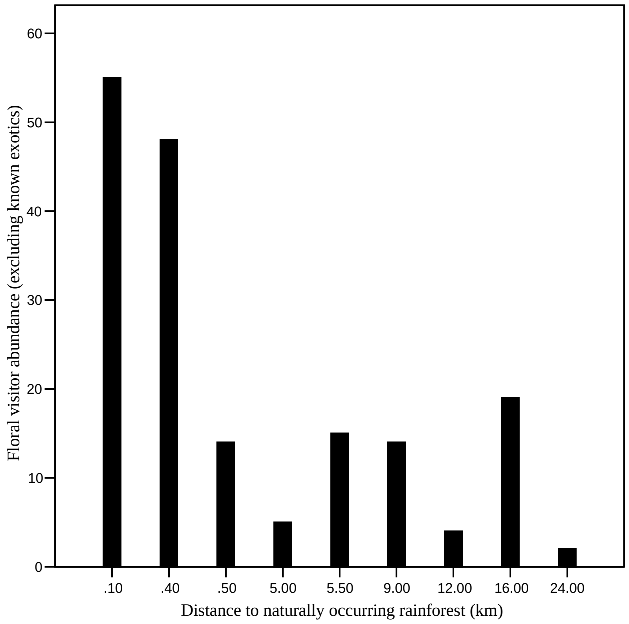

Figure 6.1. Absolute abundance of floral visitors (excluding known cosmopolitan species) in custard apple orchards located at various distances to naturally occurring rainforest.

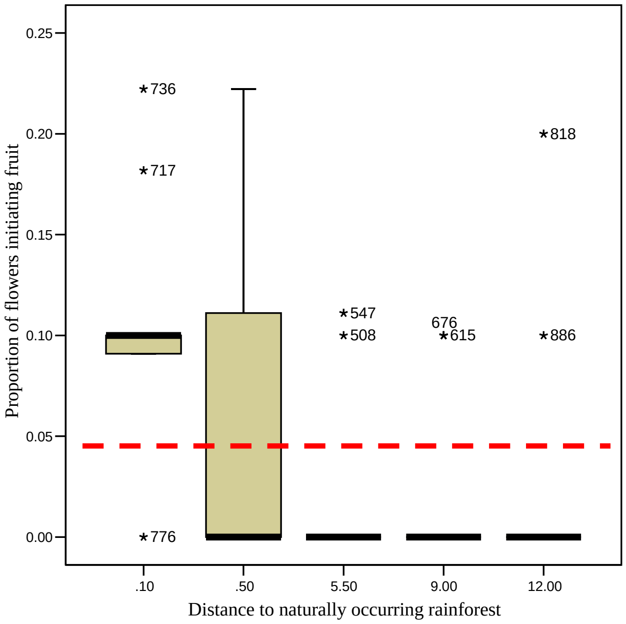

Figure 6.2. Proportion of flowers initiating a fruit in orchards located at various distances to naturally occurring rainforest. Dashed red line indicates the maximum natural fruit set for other custard apple growing regions.

This decline in fruit productivity was due to pollen limitation in orchards across the landscape gradient (isolated from naturally occurring rainforest) because flowers that were supplemented with pollen in these orchards produced the same if not more fruit than those located in close proximity to naturally occurring rainforest.

Although custard apple productivity is low, fruit productivity in the orchard located at 100 metres from naturally occurring rainforest exceeded fruit production rates from open pollinated flowers in other commercial growing regions (George *et al* 1989).

I think it is unwise to dismiss the results of this empirical work based on the low overall productivity of this crop but rather focus on the similarity among results of studies that have found negative relationships between floral visitor species richness and abundance relative to orchard isolation (Steffan- Dewenter & Tscharntke 1999, Steffan-Dewenter *et al* 2002, Ricketts 2004). These results all indicate that crop isolation resulting from reduced area of naturally occurring habitats will negatively affect both the richness and abundance of pollinator species in a landscape and the productivity (fruit initiation) of resulting crops, implying that a threshold area of natural habitat in a landscape could be reached beyond which crop production resulting from naturally derived pollinators may no longer be sustainable.

Consequences of crop expansion on pollinator species richness and abundance and the resulting fruit and seed production in agricultural landscapes are rarely considered during decisions on crop expansion, representing an unvalued cost to both the grower and the landscape. The cost to growers is manifest through a reduction in productivity and therefore market value of a resulting crop that will be born ultimately by the consumer. In the landscape context, on the Atherton Tablelands, north Queensland, rainforest occurs in isolated remnants and it is likely that dispersal of many floral visitor species would not occur between rainforest patches separated by distances greater than 400 metres, limiting foraging opportunities and pollen movement to within rainforest patch plant species and individuals; reducing genetic mixing between plant con-specifics.

The effect of pollen source (cu. ‘Hillary White’ vs. cu. ‘African Pride’) on fruit production in hand-pollinated flowers has application to growers who necessarily use this technique in the absence of sufficient pollination services by arthropods. Hand-pollinating flowers with pollen sourced from ‘African Pride’ cultivar trees will increase yield in terms of the quality of fruits produced (larger mass and better shape); both measures that are considered valuable in the marketplace. Hand pollination is such a laborious practice that any methods to improve fruit yield should be well received by custard apple growers.

REFERENCES

A Regional Profile: Atherton Tablelands Region, Queensland. 2002. Department of Transport and Regional Services, Canberra.

Agriculture in tropical north Queensland, Atherton Tablelands. 2004. Advance Cairns. http://www.advancecairns.com/profiles/at.htm

Ackerman, J. D., and A. M. Montalvo. 1990. Short and long-term limitations to fruit production in a tropical orchid. *Ecology* 71.

Ahmed, M. S. 1936. Pollination and selection in *Annona squamosa* and *A. cherimoya*. Egypt Min. Agr. Tech. and Sci. Serv., Hort. Sect. 157.

Aizen, M. A., and P. Feinsinger. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology*. 75(2). 1994. 330-351.

Allen-Wardell, G., G. P. Bernhardt, A. Bitner, R. Burquez, S. Buchmann, J. Cane, P. A. Cox, V. Dalton, P. Feinsinger, M. Ingram, D. Inouye, C. E. Jones, K. Kennedy, P. Kevan, H. Koopowitz, R. Medellin, S. Medellin-Morales, and G. P. Nabhan. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* 12:8-17.

Altieri, M.A. 2000. The ecological impacts of transgenic crops on agroecosystem health. *Ecosystem Health* 6:13-23.

Armstrong, J. A. 1979. Biotic pollination mechanisms in the Australian flora - a review. *New Zealand Journal of Botany* 17:467-508.

Arreguin-Sanchez, F. 2001. Towards the management of fisheries in the ecosystem context: the case of Mexico. *US Fish Bull. Coop.* 14 (1-4): 4-12.

Ayre, D. J., and R. J. Whelan. 1989. Factors controlling fruit set in hermaphroditic plants and the contribution of studies with the Australian Proteaceae. *Trends in Ecology and Evolution* 4:267-272.

Bartareau, T. 1995. Pollination limitation, costs of capsule production and the capsule-to-flower ratio in *Dendrobium monophyllum* F.Muell. (Orchidaceae). *Australian Journal of Ecology* 20:257-265.

Benitez-Malvido, J., and M. Martinez-Ramos. 2003. Influence of edge exposure on tree seedling species recruitment in tropical rain forest fragments. *Biotropica* 35:530-541.

Blanche, K.R., Anderson, A.N., Ludwig, J.A. 2001. Rainfall-contingent detection of fire impacts: responses of beetles to experimental fire regimes. *Ecological Applications* 11(1): 86-96.

Blanche, R., R. Bauer, S. Cunningham, and R. Floyd. 2002. Services and dis-services of rainforest insects to crops in north Queensland. Cooperative Research Centre for Tropical Rainforest Ecology and Management, Cairns.

Blanche, R. and S. Cunningham. 2005. Rain forest provides pollinating beetles for Atemoya crops. *Horticultural Entomology* 98(4): 1193-1201.

Bosch, J., and W. P. Kemp. 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. *Bulletin of Entomological Research* 92:3-16.

Brooks, K. N. 1997. Hydrology and the management of watersheds, 2nd ed edition. Iowa State University Press, Ames, Iowa.

Buchmann, S. L., and G. P. Nabhan. 1996. The Forgotten Pollinators. Island Press, Washington, D.C.

Carthew, S. M. 1993. An assesment of pollinator visitation to *Banksia spinulosa*. *Australian Journal of Ecology* 18:257-268.

Cassells, D. S., Bonell, M., Gilmour, D.A. and Valentine, P.S. 1986. Conservation and management of Australia's tropical rainforests: local realities and global responsibilities. Paper prepared for an Ecological Society of Australia Symposium on The Ecology of Australia's Wet Tropics, University of Queensland, 25-28 August 1986. 34 pp

Cork, S. J., and D. Shelton. 2000. The Nature and Value of Australia's Ecosystem Services: A Framework for Sustainable Environmental Solutions. Pages 151-159 in 3rd Queensland Environmental Conference. Environmental Engineering Society, Queensland Chapter, The Institution of Engineers, Australia, Queensland Division, and Queensland Chamber of Commerce and Industry, Queensland.

Constanza, R, Ralph d'Arge, Rudolf de Groot, Stephen Farber, Monica Grasso, Bruce Hannon, Karin Limburg, Shahid Naeem, Robert O'Neill, Jose Paruelo, Robert Raskin, P. Sutton, and M. v. d. Belt. 1987. The value of the world's ecosystem services and natural capital. *Nature* 387:253-260.

Cunningham, S. A. 2000. Depressed pollination in habitat fragments causes low fruit set. *Proceedings of the Royal Society of London - Series B: Biological Sciences*. 267:1149-1152.

Cunningham, S. A., F. FitzGibbon, and T. A. Heard. 2002. The future of pollinators for Australian agriculture. *Australian Journal of Agricultural Research* 53:893-900.

Daily, G. C. 1997. Nature's services : societal dependence on natural ecosystems. Island Press, Washington, D.C.

Debinski, D. M., and R. D. Holt. 2000. A survey and overview of habitat fragmentation experiments. *Conservation Biology*. 14(2), 342-355.

De Marco Jr.P and F.M. Coelho. 2004. Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity and Conservation* 13: 1245-1255.

Erwin, T. L. 1983. Beetles and other Arthropods of the tropical forest canopies at Manaus, Brazil, samples with insecticidal fogging techniques. Pages 59-75 in S. L. Sutton, T. C. Whitmore, and A. C. Chadwick, editors. Tropical Rain Forests: Ecology and Management. Blackwell Scientific Publications, Oxford.

Fearnside, P. M. 2000. Global warming and tropical land-use change: Greenhouse gas emissions from biomass burning, decomposition and soils in forest conversion, shifting cultivation and secondary vegetation. *Climatic Change*. 46(1-2): 115-158.

Free, J. B. 1993. Insect pollination of crops. Academic Press, London.

Gazit, S., I. Galon, and H. Podolor. 1982. The role of nitidulid beetles in natural pollination of annona in Israel. *Journal of the American Society for Horticultural Science* 107:849-852.

George, A. P., and R. J. Nissen. 1988. The effects of temperature, vapor pressure deficit and soil moisture stress on growth, flowering and fruit set of custard apple (*Annona cherimola* X *Annona squamosa*) cultivar African Pride. *Scientia Horticulturae* 34:183-192.

George, A. P., R. J. Nissen, D. A. Ironside, and P. Anderson. 1989. Effects of nitidulid beetles on pollination and fruit set of *Annona* spp. hybrids. *Scientia Horticulturae* 39:289-299.

George, A. P., R. J. Niessen, and J. A. Campbell. 1992. Pollination and selection in annona species (cherimoya, atemoya, and sugar apple). *Acta Horticulturae* 321:178-185.

Ghazoul, J. 2005. Buzziness as usual? Questioning the global pollination crisis. *Trends in Ecology and Evolution* 20(7): 367-373.

Goldingay, R. L. 1988. Further assessment of pollen limitation in the waratah (*Telopea speciosissima*). *Australian Journal of Botany* 48.

Gottsberger, G. 1974. The structure and function of the primitive angiosperm flower-a discussion. *Acta bot. neerl.* 23:461-471.

Gottsberger, G. 1999. Pollination and evolution in neotropical Annonaceae. *Plant Species Biology* 14:143-152.

Goulder, L. H., and D. Kennedy. 1997. Valuing Ecosystem Services: Philosophical Framework and Empirical Approaches in G. C. Daily, editor. Nature's Services: Societal Dependance on Natural Ecocsystems. Island Press, Washington, D.C.

Goulson, D., and L. C. Derwent. 2004. Synergistic interactions between an exotic honeybee and an exotic weed: pollination of *Lantana camara* in Australia. *Weed Research* 44:195.

Gross, C. L., and D. Mackay. 1988. Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). *Biological Conservation* 86:169-178.

Hardin, G. 1968. The Tragedy of the Commons. *Science* 162:1243-1248.

Harris, L. D. 1984. The fragmented forest : island biogeography theory and the preservation of biotic diversity. University of Chicago Press, Chicago.

Heard, T. A. 1993. Pollinator requirements and flowering patterns of *Macadamia integrifolia*. *Australian Journal of Botany* 41:491-497.

Heard, T. A., and E. M. Exley. 1994. Diversity, Abundance and Distribution of Insect Visitors to Macadamia Flowers. *Environmental Entomology* 23:91-100.

Heard, T. A. 1999. The Role of Stingless Bees in Crop Pollination. *Annu. Rev. Entomol*. 44:183-206.

Hilbert, D. W., N. Roulet, and T. Moore. 2000. Modelling and analysis of peatlands as dynamical systems. *Journal of Ecology* 88:230-242.

Hilbert, D. W. 2002. Non-linear Systems in H. A. Mooney and J. G. Canadell, editors. The Earth System: biological and ecological dimensions of global environmental change. John Wiley & Sons, Ltd., Chichester. pp 450-455.

Hill, J. 2003. Pollination Processes in the Rainforest Understorey Trees *Pseudovaria froggattii* and *P. mulgraveana*. BSc with Honours. James Cook University, Cairns.

Hyland, B. P. M., T. Whiffin, D. C. Christophel, B. Gray, R. W. Elick, and A. J. Ford. 1999. Australian Tropical Rainforest Trees and Shrubs. An interactive identification system for trees and shrubs. CSIRO Publishing, Collingwood, Victoria, Australia.

Irvine, A. K., and J. E. Armstrong. 1988. Beetle pollination in Australian tropical rainforests. *Proceedings of the Ecological Society of Australia* 15:107-113.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado, Niwot, CO, USA.

Kearns, C. A., D.W. , D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29.

Kenmore, P., and R. Krell. 1998. Global perspectives on pollination in agriculture and agroecosystem management. in International Workshop on the Conservation and Sustainable Use of Pollinators in Agriculture, with Emphasis on Bees, Sao Paulo, Brazil.

Kershaw, P. 1974. A long continuous pollen sequence from north-eastern Australia. *Nature* 251:222-223.

Kevan, P. G., and T. P. Phillips. 2002. The Economic Impacts of Pollinator Declines: An Approach to Assessing the Consequences. *Conservation Ecology* 5.

Klein, A.M., I. Steffan-Dewenter, D. Buchori, and T. Tscharntke. 2002. Effects of land-use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps. *Conservation Biology* 16:1003-1014.

Klein, A.-M., I. Steffan-Dewenter, and T. Tscharntke. 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London - Series B: Biological Sciences* 270:955-961.

Kremen, C. and T. Ricketts. 2000. Global perspectives on pollination disruptions. *Conservation Biology* 14(5): 1226-1228.

Kremen, C., N. M. Williams, and R. W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America* 99:16812-16816.

Kremen, C., Williams, N.M., Bugg, R.L., Fay, J.P. and Thorp, R.W. 2004. The area of requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters* 7: 1109-1119.

Kruess, A., and T. Tscharntke. 2000. Species richness and parasitism in a fragmented landscape: Experiments and field studies with insects on Vicia sepium. *Oecologia*. 122(1): 129-137.

Laurance, W. F., and R. O. Bierregaard. 1996. Fragmented Tropical Forests. *Bulletin of the Ecological Society of America* 77.

Laurance, W. F., T. E. Lovejoy, H. L. Vasconcelos, E. M. Bruna, R. K. Didham, P. C. Stouffer, C. Gascon, R. O. Bierregaard, S. G. Laurance, and E. Sampaio. 2002. Ecosystem decay of amazonian forest fragments: A 22-year investigation. *Conservation Biology* 16:605-618.

Lee, T. D. 1988. Patterns of fruit and seed production in J. W. Lovett Doust, editor. Plant Reproductive Ecology. Oxford University Press, Oxford, UK. pp 179-199.

Loreau, M., N. Mouquet, and R. D. Holt. 2003. Meta-ecosystems: a theoretical framework for a spatial ecosystem ecology. *Ecology Letters* 6.

Lovei, G. L., and M. Cartellieri. 2000 Ground beetles (Coleoptera, Carabidae) in forest fragments of the Manawatu, New Zealand: Collapsed assemblages? SO - *Journal of Insect Conservation.* 4(4): 239-244.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, N.J.

Malcolm, D. T., B. K. A. Nagel, I. Sinclair, and I. J. Heiner. 1999. Soils and agricultural land suitability on the Atherton Tablelands. Dept. of Natural Resources, Queensland, Australia.

Mayfield, M. M., N. M. Waser, and M. V. Price. 2001. Exploring the `Most Effective Pollinator Principle' with Complex Flowers: Bumblebees and *Ipomopsis aggregata*. *Annals of Botany* 88:591-596.

McGregor, S. E. 1976. Insect Pollination of Cultivated Crop Plants. USDA Agriculture Handbook Vol 496.

Michael Proctor, P. Yeo, and A. Lack. 1996. The natural history of pollination. Harper Collins, London.

Momose, K., T. Yumoto, T. Nagamitsu, M. Kato, H. Nagamasu, S. Sakai, R. D. Harrison, T. Itioka, A. A. Hamid and T. Inoue. 1998. Pollination biology of a lowland dipterocarp forest I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. *American Journal of Botany* 85:1477-1501.

Monteith, G. B. 1973. Entomological notes: Dung. beetles as pollinators of an Arum. Lily. *News. Bull. Entomol. Soc. Queensland* 97:13.

Mooney, H. A., and P. R. Ehrlich. 1997. Ecosystem services: a fragmented history. in Nature's services : societal dependence on natural ecosystems. Island Press, Washington, D.C.

Morrisen, A., D. Astridge, V. Hansen, and R. Elder. 2003. DPI note: hawk moth pollinators in papaya.

Motten, A. F., D. R. Campbell, D. E. Alexander, and H. L. Miller. 1981. Pollination effectiveness of specialist and generalist visitors to a North Carolina population of *Claytonia virginica*. *Ecology* 62:1278-1287.

Mustajarvi, K., P. Siikamaki, S. Rytkonen, and A. Lammi. 2001. Consequences of plant population size and density of plant pollinator interactions and plant performance. *Journal of Ecology* 89:8087.

Myers, N. 1996. Environmental services of biodiversity. *Proceedings of the National Academy of Sciences of the United States of America.* 93(7): 2764-2769.

Nadel, H. 1990. Beetle pollination of sugar apples and atemoyas or: why a rotten fruit is not all bad. *Tropical Fruit News*. pp.4-5

Nadel, H., and J. E. Pena. 1994. Identity, behavior, and efficacy of nitidulid beetles (Coleoptera: Nitidulidae) pollinating commercial *Annona* species in Florida. *Environmental Entomology* 23:4.

Nagamitsu T, I. T. 1997. Cockroach pollination of *Uvaria elmeri* (Annonaceae) in a lowland mixed-dipterocarp forest in Sarawak. *American Journal of Botany* 84:208-213.

Nagel, J., J. E. Pena, and D. Habeck. 1989. Insect pollination of atemoya in Florida. *Florida Entomologist* 72:207-211.

State of the world's forests 2001. Food and Agriculture Organization of the United Nations, Rome, Italy.

Pearce, D. W., and D. Moran. 1994. The Economic Value of Biodiversity. Earthscan Publications, London.

Peña, J. E., A. Castineiras, R. Bartelt, and R. Duncan. 1999. Effect of pheremone bait stations for sap beetles (Coleoptera: Nitidulidae) on *Annona* spp. fruit set. *Florida Entomologist* 82:475-480.

Perfecto Vvette, J. V., P. Hanson, and V. Cartin. 1997. Arthropod biodiversity loss and the transformation of a tropical agro-ecosystem. *Biodiversity & Conservation*. 6:935-945.

Piessens, K., O. Honnay, K. Nackaerts, and M. Hermy. 2004. Plant species richness and composition of heathland relics in north-western Belgium: evidence for a rescue-effect? *Journal of Biogeography* 31:1683-1692.

Potts, S. G., A. Dafni, and G. Ne’eman. 2001. Pollination of a core flowering shrub species in Mediterranean phrygana: variation in pollinator diversity, abundance and effectiveness in response to fire. *Oikos* 92.

Rainforest Conservation Society of Queensland, A. H. C. 1986. Tropical rainforests of North Queensland : their conservation significance / a report to the Australian Heritage Commission by the Rainforest Conservation Society of Queensland. Australian Government Publishing Service, Canberra.

Raven, P. H., R. F. Evert, and S. E. Eichhorn. 2003. The Biology of Plants. W.H. Freeman and Company Publishers, New York, USA.

Richards, A. J. 2001. Does Low Biodiversity Resulting from Modern Agricultural Practice Affect Crop Pollination and Yield? *Annals of Botany* 88:165-172.

Ricketts, T. H. 2002. Coffee pollination in fragmented tropical landscapes: Conservation of an important ecosystem service. *Ecological Society of America Annual Meeting Abstracts*:246.

Ricketts, T. H., G. C. Daily, P. R. Ehrlich, and C. D. Michener. 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences of the United States of America* 101:12579-12582.

Rosen, C. 2000. World resources 2000-2001, people and ecosystems: the fraying web of life. Elsevier Science, Kidlington, U.K.

Rosenzweig, M. L. 1995. Species diversity in space and time. Cambridge University Press, Cambridge, UK.

Roubik, D. W. 1995. Pollination of cultivated plants in the tropics. 118, Food and Agriculture Organisation of the United Nations, Rome.

Salati, E., and P. B. Vose. 1984. Amazon Basin: A System in Equilibrium. *Science* 225:129-138.

Salzman, J. 1998. Ecosystem Services and the Law. *Conservation Biology* 12:497.

Schroeder, C. A. 1995. Pollination strategy in cherimola. Proceedings from the Sixth Conference of the Australian Council on Tree and Nut Crops Inc, Lismore, Australia. http://www.newcrops.uq.edu.au/acotanc/papers/schroede.htm

Silberbauer-Gottsberger, I., G. Gottsberger, and A. C. Webber. 2003. Morphological and functional flower characteristics of new and old world Annonaceae with respect to their mode of pollination. *Taxon* 52:701-718.

Snow, A. A., and D. F. Whigham. 1989. Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). *Ecology* 70:1286-1293.

Steffan-Dewenter, I., U. Muenzenberg, and T. Tscharntke. Pollination, seed set and seed predation on a landscape scale. *Proceedings of the Royal Society of London - Series B: Biological Sciences*. 268:1685-1690.

Steffan-Dewenter, I., and T. Tscharntke. 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121:432-440.

Steffan-Dewenter, I., and T. Tscharntke. 2002. Insect communities and biotic interactions on fragmented calcareous grasslands: A mini review. *Biological Conservation* 104:275-284.

Steffan-Dewenter, I., U. Muenzenberg, C. Buerger, C. Thies, and T. Tscharntke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* 83:1421-1432.

Stork, N. E., D. S. Srivastava, A. D. Watt, and T. B. Larsen. 2003. Butterfly diversity and silvicultural practice in lowland rainforests of Cameroon. *Biodiversity & Conservation* 12:387-410.

Terry, I. 2001. Thrips: The primeval pollinators? Pages 157-162 in R. Marullo and L. Mound, editors. Thrips, Plants, Tospoviruses: The Millennial Review. CSIRO Entomology, Reggio Calabria, Italy.

Thakur, D. R., and R. N. Singh. 1965. Studies on pollen morphology, pollination and fruit set in some annonas. *Indian Journal of Horticulture* 22:10-18.

Thies, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Effects of landscape context on herbivory and parasitism at different spatial scales. *Oikos* 101:18-25.

Thomson, J. D., and K. Goodell. 2001. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology* 38:1032-1044.

Tracey, J. G., and L. J. Webb, 1975. 1975. Key to the vegetation of the humid tropical region of North Queensland, with 15 maps at 1:100,000 scale. in. CSIRO Long Pocket Laboratories, Indooroopilly, Queensland.

Tracey, J. G. 1982. The vegetation of the humid tropical region of North Queensland. CSIRO, Melbourne.

Van der Pijl, L. 1969. Principles of Dispersal in Higher Plants. Springer-Verlag, New York.

Vitousek, P. M., J. D. Aber, R. H. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Source and consequences. *Ecological Applications* 7(3): 737-750.

Voyiatzis, D. G., and G. Paraskevopoulou-Paroussi. 2002. Factors affecting the quality and in-vitro germination capacity of strawberry pollen. *Journal of Horticultural Science & Biotechnology* 77:200-203.

Wallace, H. M., and L. S. Lee. 1999. Pollen source, fruit set and xenia in mandarins. *Journal of Horticultural Science & Biotechnology* 74:82-86.

Wallace, H. M., B. J. King, and L. S. Lee. 2002. Pollen flow and the effect on fruit size in an 'Imperial' mandarin orchard. *Hortscience* 37:84-86.

Webber, A. C., and G. Gottsberger. 1995. Floral biology and pollination of *Bocageopsis multiflora* and *Oxandra euneura* in Central Amazonia with remarks on the evolution of stamens in Annonaceae. *Feddes Repertorium* 106:515-524.

Westerkamp, C., and G. Gottsberger. 2000. Diversity pays in crop pollination. Crop *Science* 40:1209-1222.

Williams, G. P., and L. A. Adam. 1994. A review of rainforest pollination and plant–pollinator interactions with particular reference to Australian subtropical rainforests. *Australian Zoologist* 29:177-212.