Reproductive biology of *Canna* species naturalized in southern Africa

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ABSTRACT

Invasive species have many deleterious effects, including ecological and economic impacts. Invasiveness of exotic plant species has been linked to various attributes, including reproductive traits such as potential for uniparental reproduction, co-option of pollinators in the invasive range, and hybridization with congeners. Therefore, studies of the reproductive biology of invasive plant species can provide invaluable information about the risks species may pose and assist in their management. Several taxa of *Canna* L. native to the Americas have been introduced into South Africa, but their modes of reproduction have remained largely unknown. In this study, I investigated the breeding systems, extent of inbreeding depression, pollinator assemblages, pollinator effectiveness, extent of pollen limitation, and hybridization potential in populations of *Canna indica* and *Canna glauca* in KwaZulu-Natal, South Africa. Results showed that *C. indica* is partially autogamous and self-compatible, whereas *C. glauca* is allogamous, but the orange-flowered form (OF) of this species is partially self-compatible while the yellow-flowered form (YF) of this species is fully self-compatible. Inbreeding depression was detected in self-fertilized progeny of *C. indica*, but not in *C. glauca* OF. I found that seeds of *C. indica* are sired readily by *C. glauca* YF and partially by *C. glauca* OF, and the two colour forms of *C. glauca* are completely inter-fertile. Despite the apparent adaptation for pollination by birds, I found that honeybees are the most frequent and important pollinators of all taxa. Sunbirds rob flowers of nectar without effecting pollination. In *C. indica*, I found that honeybees and social bees are frequent visitors. Honeybee pollination in *C. indica* resulted in significantly higher seed set than did autonomous self-pollination. Both forms of *C. glauca* are pollinated effectively by pollen-collecting honeybees. I found that *C. indica* experienced pollen limitation in two of the three years of study. *Canna glauca* YF experienced severe pollen limitation in one year of the study, whereas *C. glauca* OF did not experience pollen limitation in any of the study years. Both study taxa exhibit vegetative reproduction. The results of this study highlight the potential for honeybees to promote reproduction of invasive plant species adapted to other pollinators (primarily hummingbirds in this case). While *C. indica* is already a declared invasive species, this study suggests that *C. glauca* should also be prioritized in management programs due to its prolific seed production resulting from co-option of local bee pollinators, absence of inbreeding depression, and ease of hybridization with other species.

PREFACE

The research described in this thesis was carried out at the School of Life Sciences, University of KwaZulu-Natal (Pietermaritzburg), from January 2016 to September 2018; however, data from 2014 and 2015 was used, under the supervision of Professor Steven Johnson (University of KwaZulu-Natal).

The work presented in this thesis represents the original work of the author and has not been otherwise submitted in any other form for any degree or diploma to any other University. Where use has been made of the work of others, this has been duly acknowledged in the text.

Signed:

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Mvana Simon Sibiya (Candidate)

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Professor Steven D. Johnson (Supervisor)

COLLEGE OF SCIENCE AND AGRICULTURE DECLARATION 1 – PLAGIARISM

I, Mvana Simon Sibiya, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Mvana Simon Sibiya

September 2018

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Chapter 1

Invasive alien plant species are a major threat to biodiversity, economy, water sources, human and animal well-being, as well as ecosystem services provided to human societies in areas infested with these species (Rouget et al., 2004, Stout et al., 2006, Forsyth et al., 2012, Ward et al., 2012, Kumschick et al., 2017a, Latombe et al., 2017, Mafanya et al., 2017, Visser et al., 2017). Invasive alien plant species may affect biodiversity of infested ranges in many different ways (Kumschick et al., 2017a). For example, these plants may disturb prevailing disturbance regimes by promoting or suppressing fire (Foxcroft et al., 2008, Mafanya et al., 2017); they may usurp copious amounts of water, light, and oxygen for native species (Foxcroft et al., 2008, Abella et al., 2009); they may also modify processes of nutrient cycling, disturb long standing mutualisms, and donate limiting resources that prevent proliferation of native species (Foxcroft et al., 2008, Stout et al., 2006). The exotic annual grass *Bromus rubens* (Poaceae) in the western U.S.A., for example, has encouraged wildfires that kill many native long-lived plants such as *Yucca brevifolia* (Asparagaceae) - in turn these wildfires increase soil erosion and lower the quality of breathable air (Abella et al., 2009). Indeed, in the Americas, Australia, and many tropical islands, grasses generally modify ecosystems by disturbing prevailing fire regimes (Visser et al., 2017). Invasive grasses not only encourage fires. The African grass species *Eragrostis plana* Nees (Poaceae), for example, in Brazil, outcompeted and displaced the native *Aristida* species (Poaceae) that were used for cattle grazing in pastures (Zenni and Ziller, 2011). In its invasive ranges such as Britain and Ireland, *Rhododendron ponticum* L. (Ericaceae) shades out native species and limits their growth and in addition this species is also thought to suppress proliferation of native species through donation of allelopathic acids (Stout et al., 2006). *Lantana camara* L. (Verbenaceae), the notorious invasive plant from the Old World tropics, has been reported to invade a staggering 40 000 km^2 of forest biome in Australia that includes national parks (Hanley and Goulson, 2003). Cacti are among the most abundant invasive plants in southern Africa (e.g., 35 species listed as invaders in the NEM: BA regulations) and many reproduce vegetatively so that they form dense stands with 100 % canopy cover disturbing light regime to native vegetation (Kaplan et al., 2017). The invasive salt cedar *Tamarix ramosissima* (Tamaricaceae) in the arid western U.S.A., following the formation of dense stands, consumes considerable amounts of water through deep-root systems and high leaf areas when compared to the lower leaf area native flora in the riparian areas it invades (Abella et al., 2009).

Invasive alien plant species not only raise concerns associated with environmental impacts as cited above, but also economic impacts (Hong et al., 2008, Kumschick et al., 2017a, Kumschick et al., 2017b). It has been estimated that invasive alien plant species in the United States, Canada, Britain,

and India exacted a combined total cost of US$ 336 billion per year in overall impacts associated with ecosystem services (Semenya et al., 2012). *Bromus rubens* (see above) fuels wildfires in the arid west of U.S.A., wildfires estimated to cost millions of dollars when measures to control them are undertaken (Abella et al., 2009). In Australia, control of *L. camara* costs approximately A$ 10 million per year, whereas the livestock industry from this *L. camara* invasion has suffered losses of A$ 7.7 million due to reduced stocking densities and poisoning of approximately 1500 cattle annually that led to their death (Hanley and Goulson, 2003). In general, according to 2004 reports, Australia expends an estimate of AU$ 4.4 billion per year in the control of invasive alien plant species and associated agricultural loss costs (Ward et al., 2012). *Tamarix ramosissima*, the deep-rooted invasive alien tree in the riparian areas of western U.S.A., has had economic costs that were estimated to be US$ 133 – 285 million annually, and these costs were associated with agricultural losses and other decreases in resource values due to its nature of forming dense, monospecific stands that consume large quantities of water (Abella et al., 2009). In southern Africa, invasive alien plant species alone have been estimated to cost an overwhelming R 6.5 billion per year to control (Wilson et al., 2013). Therefore, owing to the impacts of invasive alien plant species, it is expedient and cost-effect to assess potential invasiveness of a species based on its biology, ecology, and population dynamics in order to provide information needed for managing the species as well as to gain insights about the risks a species may pose (Stout et al., 2006, Van Wilgen et al., 2007, Le Roux et al., 2010, Kaplan et al., 2012). Invasive alien plant species may impose dramatic negative effects on infested ecosystems (Hooper et al., 2005, Van Wilgen et al., 2007, Wilson et al., 2013) and such invasive plants could ultimately contribute to local extinctions of some native plant species (Hooper et al., 2005, Traveset and Richardson, 2006), as it has been demonstrated by Vila and Weiner (2004) that invasive species are generally more competitive than native species.

The history of introduction of plants to new ranges around the world by humans

Anthropogenic activities around the world have been, and are still today, changing the biotic structure and composition of ecological communities locally, regionally, nationally and globally (Hooper et al., 2005). One of the often noted activity of humans that has been considerably important in bringing global scale changes to the ecological communities of the world (Hanley and Goulson, 2003) is the introduction of exotic species to habitats new to them (Parker, 1997, Heywood, 2001, Foxcroft et al., 2008, Abella et al., 2009, Zenni et al., 2009). The introduction, domestication, cultivation, and tending of wild plant species for ornamentation and agricultural purposes begun with

some few early human civilizations around 2100 B.C. – 1600 B.C. (Heywood, 2001, Novoa et al., 2017). The cultivation and tending of unimproved wild plant species for ornamentation continued long after the Xia Dynasty (Heywood, 2001). Cultivating wild plants for ornamentation occurred in civilizations from around the subtropics of Africa, Asia, and temperate zones of Europe (Novoa et al., 2017). According to literature (Rejmanek and Richardson, 1996, Parker, 1997, Richardson et al., 2000, Heywood, 2001, Abella et al., 2009, Zenni et al., 2009, Novoa et al., 2017), exotic plant species have been disseminated widely by man across the world, to an extent that even protected areas are inhabited by alien species introduced by humans (Foxcroft et al., 2008). As a consequence of these human-mediated introductions of alien species and alterations of biotic environments, the structure and composition of communities change dramatically (Kumschick et al., 2017b) and hence disruptions in ecosystems functioning are inevitable (Hooper et al., 2005, Dohzono and Yokoyama, 2010, Zengeya et al., 2017).

Why exotic plant species were introduced to different parts of the world?

Many exotic plant species were intentionally distributed to many different parts of the world so that they could provide some value to humans (Foxcroft et al., 2008, Canavan et al., 2016, Marr et al., 2017). Most pine species, for example, provide significant economic values and have been deliberately introduced to almost all countries with climates considerably matching their native ranges (Rejmanek and Richardson, 1996). Species such as *Senna didymobotrya* Mill. (Fabaceae) provide green manure in tropical Asia and America (Jaca and Condy, 2017) whereas *Opuntia ficus-indica* (Cactaceae) provides fodder for livestock in South Africa (Kaplan et al., 2017). Other introduced species valuable in agriculture include bamboo which has met increased popularity - 2.5 billion people use this group of plants (Canavan et al., 2016). Species were not only introduced for agricultural purposes, but also for stabilization of dunes, control of erosion, and other purposes (Hooper et al., 2005, Le Roux et al., 2010, Wang et al., 2013, Zengeya et al., 2017). Of the many exotic plant species introduced by man to many regions across the world, a large proportion of these intentional introductions were/or are for use in horticulture (Heywood, 2001, Maas-van de Kamer and Maas, 2008, Zenni et al., 2009, Geerts et al., 2013, Ugoletti et al., 2013). Indeed, the horticultural industry is said to be one of the pivotal pathways to introducing species in different parts of the world (Cronin et al., 2017). *Rhododendron ponticum*, for example, was introduced into Britain around 1763 AD and later in the century to Ireland as an ornamental plant that also provided shade for game and functioned as a wind-breaker (Stout et al., 2006). In 1839, in Britain and Ireland still, *Im-*

patiens glandulifera (Balsaminaceae) was introduced for ornamental purposes from the Himalayas (Ugoletti et al., 2013). Well-known species such as *Cytisus scoparius* (Leguminosae) (Parker, 1997) and *Acacia paradoxa* DC. (Fabaceae) (Zenni et al., 2009) were introduced in the U.S.A. and Chile as ornamentals respectively. In the U.S.A., professional and amateur breeders introduced over 100 new floricultural species between 1998 and 2008 (Foxcroft et al., 2008). Species of the family Araceae have been widely introduced throughout the world for horticulture (Moodley et al., 2017). *Hydrocleys nymphoides* (Limnocharitaceae), originally from Brazil and Venezuela, has been introduced into Australia as an ornamental plant, and into Zimbabwe and Kenya as both as an ornamental and wastewater treating plant in wetlands (Nxumalo et al., 2016). The horticultural trade industry is immense, to the extent that in the United Kingdom alone it employs roughly about 500 000 people and generates about £ 500 million per year; the Netherlands generates £ 1330 million annually through tulips whereas the U.S.A. has been reported to generate a revenue of US$ 11 billion/year (Heywood, 2001, Novoa et al., 2017). In South Africa reports estimate that 300 species of cacti are still introduced for use in horticulture (Novoa et al., 2017) and that many of these species can still be found in gardens and private collections (Kaplan et al., 2017). Other than the cacti, in 1997 South Africa was reported to generate a revenue of R 149.3 million annually through the horticultural trade industry of wild harvesting of fynbos flowers (Heywood, 2001). To date, the number of exotic species around the globe is large, with about 20 000 different plant species used for ornamental purposes (Heywood, 2001) and more than 13 000 naturalized vascular plant species occurring globally (Latombe et al., 2017) owing to the continuous introduction of plants for many various socio-economic benefits (Zengeya et al., 2017).

The introduction & naturalization of the genus Canna *in southern Africa & potential reasons for introductions*

Many alien plants, now with well-known ecological and economic impacts, have been introduced to South Africa. In efforts to mitigate the impacts, considerable research and management options have been explored and/or carried out in southern Africa in the recent past (Rouget et al., 2004). One particular genus that has been introduced and established in southern Africa and continental Africa from the New World is *Canna* L. (Rambuda and Johnson, 2004, Maas-van de Kamer and Maas, 2008), although there are no clear accounts as to when introductions of the abovementioned genus occurred, particularly in South Africa. The genus *Canna* is a group of herbaceous plants within the order Zingiberales (Baran et al., 2010, Almeida et al., 2013, Huber et al., 2013, Mishra et

al., 2013). All species of *Canna* are sturdy monocots with shoots that live for a few months following flowering (Maas-van de Kamer and Maas, 2008), and the clump of shoots may live for several years (Soares et al., 2005, Gupta et al., 2013). *Canna* is the only genus within the family Cannaceae (Prince, 2010, Glinos and Cocucci, 2011, Huber et al., 2013, Mishra et al., 2013) and it is composed of approximately 10 - 20 species (Kress and Specht, 2006, Glinos and Cocucci, 2011, Mishra et al., 2013) and more than 1000 hybrids (Prince, 2010, Gupta et al., 2013). *Canna* originate in the Americas (Soares et al., 2005, Baran et al., 2010, Glinos and Cocucci, 2011) but today species are widely distributed throughout the tropical and subtropical regions of the world (Prince, 2010, Tobar-Vargas et al., 2013) owing to their extensive use as ornamentals by the horticultural trade industries (Young, 1982, Baran et al., 2010). *Canna* have also been used as food items (Soares et al., 2005, Prince, 2010), medicinal plants, musical instruments (Mishra et al., 2013), and jewellery (Maas-van de Kamer and Maas, 2008). Again, there is no clear account for the reason of introduction of these species in southern Africa, however, it is very likely that they were introduced for ornamentation as many other countries have used these species for such purposes (Maas-van de Kamer and Maas, 2008, Prince, 2010). *Canna indica* L., is declared invasive and categorized as 'category 1 b' and 'category 1' invasive plant by the NEM: BA (National Environmental Management: Biodiversity Act) and CARA (Conservation of Agricultural Resources Act) regulations respectively (DAFF, 2001, DoEA, 2009, Wilson et al., 2013). Photographs of the species and forms of *Canna* included in this study are given in Fig. 1.
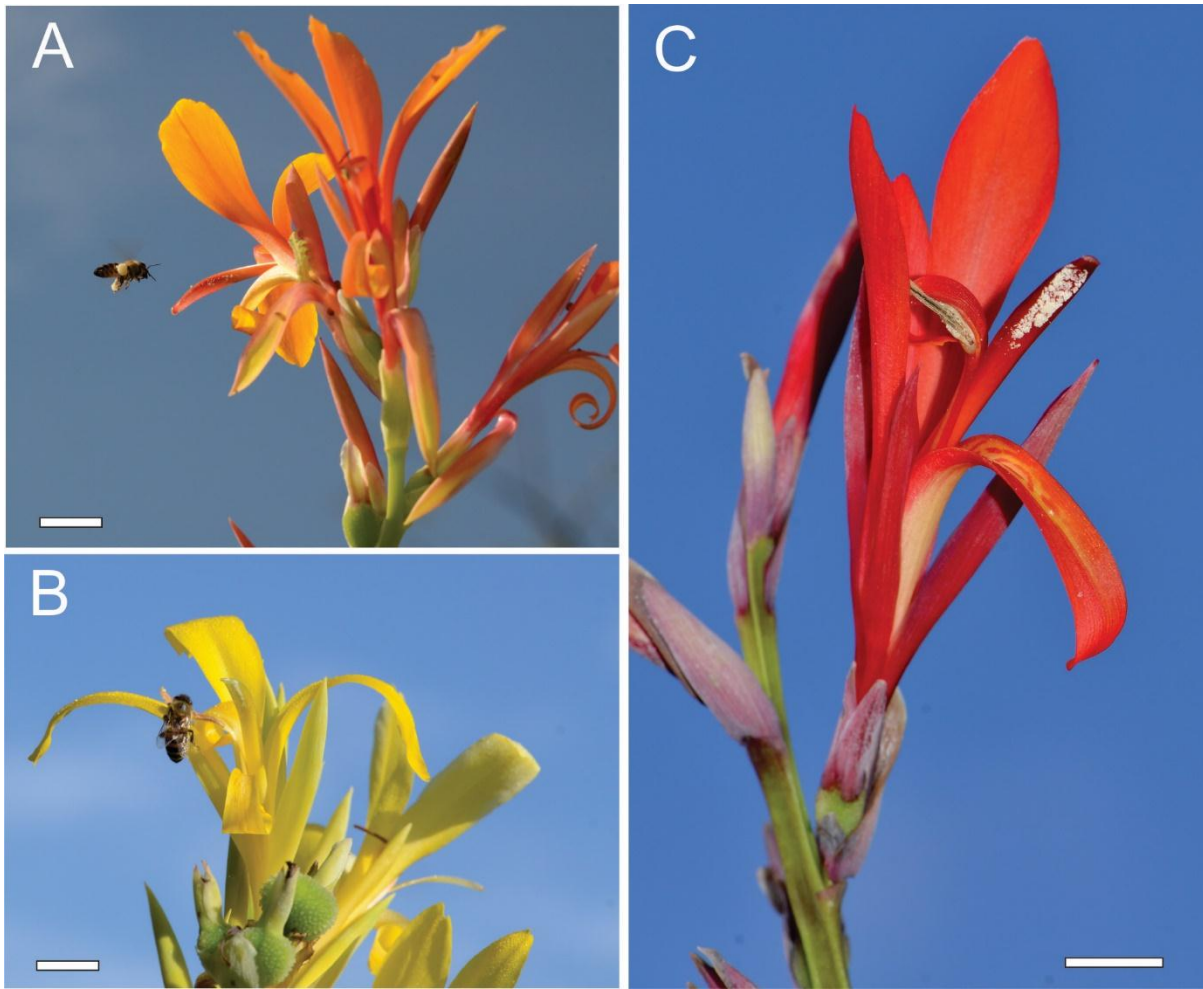
Figure 1: *Canna glauca* (orange form, hereafter *C. glauca* OF) in A and *C. glauca* (yellow form, hereafter *C. glauca* YF) in B visited by pollen-collecting honeybees. *Canna indica* L. (in C) flower with a petaloid stigma bearing pollen following a process of secondary pollen presentation while flower was in bud stage. Scale bar = 1 cm. All photos by S. D. Johnson.

Reproductive biology of **Canna** *species*

All species of *Canna* exhibit a distinct process called secondary pollen presentation (Maas-van de Kamer and Maas, 2008, Glinos and Cocucci, 2011, Maruyama et al., 2015). Secondary pollen presentation in *Canna* is categorized as "sub-terminal stylar" secondary pollen presentation, where pollen is passively loaded onto the presenting style at bud stage (Howell et al., 1993, Kubitzki, 1998, Yeo, 2012). This process occurs when the petaloid stamen wraps itself around the petaloid style in bud stage where, in ordered events, the theca deposits all of the pollen on the style so that it can be readily available to potential pollinators (Maas-van de Kamer and Maas, 2008). Only two

species of *Canna* have been explicitly demonstrated to be pollinated by hummingbirds in their native ranges. *Canna indica*, native in Argentina, has been shown to be efficiently pollinated by *Heliomaster furcifer* (Trochilidae) which contacts the receptive petaloid stigma with pollen previously placed on its bill following a visit from another congener (Glinos and Cocucci, 2011). In a more recent study conducted in Brazil, the hermit hummingbird *Phaethornis eurynome* (Trochilidae) is the effective pollinator of *Canna paniculata* in its native range (Maruyama et al., 2015). Although the above examples provide evidence that *Canna* is bird-pollinated in their native ranges, pollinators of many species of this genus have not been documented, and previously there has been only speculation and fragmentary accounts of pollinators of these species (Armbruster et al., 1999, Maasvan de Kamer and Maas, 2008, Aizen et al., 2016). Indeed, existing studies involving *Canna* are mostly restricted to the evolutionary relationships (Kress and Stone, 1982, Kirchoff, 1983, Kress and Specht, 2006, Prince, 2010, Almeida et al., 2013, Gupta et al., 2013), characterization of biominerals (Baran et al., 2010), protection of species from natural enemies (Young, 1982, Soares et al., 2005), and assessments of species as alternatives for other food sources (Lai and Tsai, 1989, Piyachomkwan et al., 2002). Therefore, the pollination biology of many invasive species, particularly the ones occurring in unfamiliar ranges, has received little attention and it remains unclear how introduced *Canna* species reproduce in new ranges.

Breeding systems of *Canna* species are largely undocumented. In Argentina, where *Canna indica* is indigenous, it has been reported that this species is pollinator dependent (Glinos and Cocucci, 2011), but tests for self-incompatibility were not performed. Interestingly, in southern Africa, where *C. indica* is reported invasive, the species has been reported to be autogamous, capable of producing fruits and seeds autonomously with self-pollen (Rambuda and Johnson, 2004). In addition, *Canna paniculata*, native in Brazil, has been reported to be self-compatible, but incapable of automatic self-fertilization (Maruyama et al., 2015). In general, it has been presumed that all species of *Canna*, and a few other Zingiberales (Wang et al., 2005, Ma et al., 2012), are self-compatible (Maasvan de Kamer and Maas, 2008). However, it appears that some species of *Canna* do require the services of pollinators to set seeds in their native ranges and that levels of autogamy may vary among populations. Therefore, more studies of the breeding systems of *Canna* are required to test whether species are generally self-compatible and whether some are autogamous, particularly in their invasive ranges.

How do other exotic plant species manage to reproduce in new ranges following introductions: Baker's focus on plant breeding systems

Most angiosperms including self-compatible species rely on animal pollinators for successful fruit and seed set following successful out-crossing (Hanley and Goulson, 2003). However, among invasive alien plant species there is a relatively high percentage of self-compatible species, particularly autogamous species (van Kleunen and Johnson, 2007a). Notable examples of highly successful invasive alien plants that have selfing ability include *Alliaria petiolla* (Brassicaceae), *Bromus tectorum*, *Carpobrotus edulis* (Aizoaceae), and *Mesembryanthemum crystallinum* (Aizoaceae) in the United States (Richardson et al., 2000); *Bidens pilosa* (Asteraceae), *Sonchus asper* (Asteraceae), and *Sonchus oleraceus* (Asteraceae) in China (Hao et al., 2011), and *Senna didymobotrya* and *Canna indica* in southern Africa (Rambuda and Johnson, 2004). In an attempt to explain the apparent correlation between the breeding system of individuals and their colonization ability, Baker's "rule" was published which was later termed "Baker's Law" (Carr et al., 1986). Baker's original ideas were based on observations he made on freshwater shrimps (Notostraca) inhabiting the ephemeral pools. He noted that these invertebrates, although ancestrally diocious, had evolved hermaphroditism and that eggs of these shrimps were dispersed by wind or animals the same way as plant seeds. Hence, Baker noted that in animals, autogamous hermaphroditism provided opportunities for rapid establishment.

Baker's Rule is logical and tidy for a few reasons. It is logical to expect that plants introduced from their native ranges to new ranges may suffer from mate limitation (van Kleunen and Johnson, 2007a) or lack suitable pollinators in new biogeographical ranges such that their expansion potential is limited or not realized at all (Stout, 2007, Le Roux et al., 2010). Outcrossing, when possible, can be beneficial in restricting the expression of recessive deleterious alleles in homozygous individuals (e.g., inbreeding depression Barrett and Harder, 1996) and promoting evolution of invasive plants (van Kleunen and Johnson, 2007a). However, in situations of mate or pollinator limitation, reproductive assurance may likely be attained through autonomous selfing, and, less so, through facilitated selfing (Brys and Jacquemyn, 2011). Although Baker's Rule is logical in its own right, there has been considerable arguments and skepticism about this hypothesis when applied to invasive alien plant species. For example, some scientists have raised suggestions that self-fertility may be important for the maintenance of certain traits in individuals that are colonizers, rather than self-fertility being a trait to overcome the scarcity of mates during colonization (Rambuda and Johnson, 2004). Skepticism around Baker's Rule arises due to those earlier studies that identified exceptions to Baker's Rule, such as that island plants are often dioecious and that some alien invasive plant

species are self-incompatible yet able to spread (Bawa and Beach, 1983, Crawford et al., 2008, Hao et al., 2011, Pannell and Barrett, 1998, Ward et al., 2012). Despite these doubts about the applicability of Baker's Rule to plant invasions, a number of studies have demonstrated that this hypothesis holds true for plant invasions (Rambuda and Johnson, 2004, Crawford et al., 2008, Hao et al., 2011, Ward et al., 2012, van Kleunen and Johnson, 2007a, van Kleunen et al., 2007c).

The importance of self-compatibility in assuring reproduction for introduced plant species in new ranges

It has been estimated that up to 20 % of flowering plants have evolved a selfing breeding system as a sexual strategy (Barrett, 2002). Also, in the many plants that have been introduced from their native ranges to new ranges, a small percentage of these have become invasive (Stout et al., 2006, van Kleunen and Johnson, 2007b, van Kleunen et al., 2007c) and within this group of invasive plants, there is a high proportion of self-compatible species (van Kleunen and Johnson, 2007a). Self-compatibility in invasive plants may be important for a few reasons. Self-compatibility may provide an additional source of pollen to fertilize more ovules of the same individual (Barrett and Harder, 1996, Barrett, 2002) to ultimately counteract pollen limitation caused by a deposition of an insufficient number of pollen grains in cases where exotics are not fully adapted to pollinators or when pollinator visits to populations are rare due to Allee effects (van Kleunen et al., 2007a, Rambuda and Johnson, 2004). Selfing typically alleviates the need for individuals to have mating partners, and thus promotes speedy invasion of unoccupied environments (Barrett, 2002). Furthermore, self-compatibility, together with autonomous fertilization, is likely to increase seed production even if some outcrossing occurs in the presence of suitable mates and pollinators (Hao et al., 2011), and so may impart increased propagule pressure and invasiveness of plants (van Kleunen and Johnson, 2007a, van Kleunen et al., 2007c). Despite the notable benefits of self-compatibility and autonomous self-fertility in providing reproductive assurance for invasive plant species (van Kleunen and Johnson, 2007a), self-fertility is usually associated with costs of reduced plant fitness (Stout, 2007, Lin et al., 2012), inbreeding depression (Barrett and Harder, 1996, Barrett, 2002), loss or reduction of pollen export due to increased rates of self-pollination (e.g., pollen discounting Kohn and Barrett, 1994, Barrett and Harder, 1996, Barrett, 2002), and the formation of genetically similar populations (Barrett, 2002). Nonetheless, certain breeding system attributes, particularly self-compatibility and automatic self-fertilization, may contribute to the rate of spread of invasive alien plant species and

may be an important determinant of potential invasiveness (Rambuda and Johnson, 2004, van Kleunen and Johnson, 2007a, van Kleunen and Johnson, 2007b, van Kleunen et al., 2007c)**.**

The potential importance of secondary pollen presentation in invasive alien plant species

Secondary pollen presentation is said to be a reproductive strategy employed by plants to increase accuracy of pollen transfer (Howell et al., 1993, Lin et al., 2012). However, this strategy may also be beneficial for invasive alien plant species if it breaks down and becomes a selfing strategy. Secondary pollen presentation is a pollination adaptation where pollen is developmentally relocated from the conventional anthers (which produce pollen and typically function as pollen presenters Lin et al., 2012) onto another floral organ which then assumes the key role of a pollen presenting organ (Howell et al., 1993, Imbert and Richards, 1993, Hong et al., 2008). There are approximately 3 - 5 monocotyledonous and 13 – 20 dicotyledonous plant families (Hong et al., 2008, Lin et al., 2012) known to manifest secondary pollen presentation - and these plants are from taxonomically distinct groups (Howell et al., 1993). The most common floral organs participating in the process of secondary pollen presentation are the styles and stigmas (Lin et al., 2012). However, owing to the variety of floral organs employed during this process, nine different types of secondary pollen presenting systems are recognized (Howell et al., 1993). Due to the close proximity of pollen and stigmas in flowers displaying secondary pollen presentation, self-pollination may be inevitable (Lin et al., 2012). Species such as *Canna indica*, which has been reported to be autogamous in southern Africa (Rambuda and Johnson, 2004), may benefit from the close association of pollen and stigmas and may consequently spread rapidly. Although secondary pollen presentation has been hypothesized to only increase the efficiency and precision of pollen movement from presentation organs to stigmas (Lin et al., 2012), this trait among invasive alien plants may be important in promoting their spread by assuring reproduction.

The occurrence of invasive alien plant species that require pollinators and/or are self-incompatible

The percentage of angiosperms that are exclusive or facultative out-crossers is high - approximately 80 % - and within this group most rely on movement of pollen by an animal vector (e.g., birds or honeybees) from one plant to the next in order to successfully set seeds and reproduce (Richardson

et al., 2000, Hanley and Goulson, 2003, Hargreaves et al., 2010). Many introduced invasive plant species require pollen vectors for seed set. For example, *Lonicera japonica* (Caprifoliaceae) is a woody vine native to eastern Asia but also an aggressive invader of natural ecosystems in the eastern and southern U.S.A. and depends on pollen transfer from genetically distinct plants for seed set (Larson et al., 2002). *Rhododendron ponticum* and *Impatiens glandulifera* are highly invasive species in Britain and Ireland that rely on pollinating bumblebees (*Bombus* spp.) for successful fruit and seed set to ultimately establish and spread (Stout et al., 2006, Stout, 2007, Ugoletti et al., 2013). *Solanum torvum* (Solanaceae), a native plant of Central America and the Caribbean, is another example of an invasive alien species in the U.S.A. that depends on pollinators for fruit set, though it can effect seeds with self-pollen (Liu and Pemberton, 2009). The role of pollination, particularly in promoting or limiting invasions, is thus crucial and has received little attention but needs to be further investigated (Stout et al., 2006) because even self-incompatible species (e.g., 17 % of European species in the U.S) become invasive (van Kleunen and Johnson, 2007a).

Western honeybees (**Apidae**)*: their origin & subsequent introductions to other parts of the world*

Enormous amounts of literature have highlighted and documented introductions and reasons of introductions of alien plant species to new geographical ranges (Rejmanek and Richardson, 1996, Parker, 1997, Richardson et al., 2000, Foxcroft et al., 2008, Zenni et al., 2009). However, other organisms have also been introduced widely throughout much of the rest of the world for a variety of reasons (Richardson et al., 2000, Goulson et al., 2002, Hanley and Goulson, 2003, Dohzono and Yokoyama, 2010). Social bees (e.g., honeybees and bumblebees), based on the number of studies that investigated their importance in non-native plant-pollinator or native plant-pollinator mutualisms in new ranges (McQuillan and Hingston, 1999, Goulson et al., 2002, Hanley and Goulson, 2003, Dohzono and Yokoyama, 2010), are among the many species that were introduced to new geographical regions around the world. Indeed, owing to its extensive human-mediated dissemination and naturalization in many parts of the world, the honeybee is today considered to be one of the most widespread and abundant insect in the world (Hanley and Goulson, 2003).

Western honeybees (e.g., *Apis mellifera* L., Hymenoptera; Apidae) are naturally from the continental Africa, Europe (particularly in the south-east), the Middle East, and western Asia (Hanley and Goulson, 2003, Dohzono and Yokoyama, 2010). The African subspecies of honeybees (e.g., *A. mellifera scutellata* Lepeletier) are frequently found in tropical forests and savannas whereas the Euro-

pean honeybees are typically found in temperate and Mediterranean climatic zones (Hanley and Goulson, 2003). The honeybee was probably introduced to new regions around the world in the early 1800s (Gross and Mackay, 1998). Indeed, the honeybee was first introduced into Australia in 1821 (Goulson et al., 2002) and from 1863 to 2002, feral honeybees spread widely across Australia and increased in abundance (Gross and Mackay, 1998, Goulson et al., 2002). The African honeybee *A. mellifera scutellata* was first introduced to Brazil in 1957 (Hanley and Goulson, 2003). Today these social bees together with their feral populations have expanded their ranges and became established (Goulson et al., 2002) in much of Asia's landmass, North America, the southern half of South America, Australia, and much of the neotropics (Hanley and Goulson, 2003, Dohzono and Yokoyama, 2010).

The deliberate introduction of social bees throughout much of the rest of the world has been largely for improvement of commercial crop plant pollination and, to a lesser extent, honey production (Goulson et al., 2002, Hanley and Goulson, 2003, Dohzono and Yokoyama, 2010). Although these social bees were translocated for specific purposes into their new geographic ranges, published data indicates that some of these social bees have become naturalized and have had significant impacts on native plants and invasive plants (Gross, 1993, Gross and Mackay, 1998, McQuillan and Hingston, 1999, Gross, 2001, Goulson et al., 2002, Larson et al., 2002, Hanley and Goulson, 2003, Dohzono and Yokoyama, 2010).

Honeybees: their role in the fitness of native & introduced plant species

Introduced social bees may disrupt mutualistic relationships between native plants and pollinators such that introduced social bees' presence may tamper with the foraging behavior of native pollinators - e.g., the presence of introduced *Apis mellifera* (Apidae) reduced foraging trips of *Bombus occidentalis* Greene (Apidae) to five plant species (Thomson, 2004). The disruption of mutualistic relationships between native plants and pollinators by introduced social bees may ultimately result in reduced fruit and seed set of native plants (Gross and Mackay, 1998, Goulson et al., 2002, Traveset and Richardson, 2006, Dohzono and Yokoyama, 2010). Introduced social bees may also directly reduce reproductive success of native plants, both in insect and bird-pollinated systems, by becoming important pollen thieves who strip pollen from stigmas that were previously pollinated by native anthophiles (Botes et al., 2009, Dohzono and Yokoyama, 2010, Gross, 1993, Gross and Mackay, 1998). On the other hand, introduced social bees (and in this case, some introduced solitary bees)

may be important pollinators of introduced invasive plants in new ranges (Liu and Pemberton, 2009), or important pollinators of native plant species in new ranges. For example, in a study conducted in western U.S.A. in 2001, Barthell and colleagues showed that honeybees were frequent visitors to three populations of the invasive *Centaurea solstitialis* L. (Asteraceae) and exclusion of honeybees from flower heads significantly reduced seed set of this plant species when compared to flowers open to honeybees (Hanley and Goulson, 2003). In an analysis of Charles Robertson's data of pollination published as a monograph in 1929, Memmott and Waser (2002) showed that *Apis mellifera* (Apidae) visited 217 of 456 plant species, of which 12.3 % (26.7) of these species were alien; they also mention that honeybees and other alien insects may have been ancestral pollinators to these alien plants in Illinois, U.S.A. Work by Memmott and Waser (2002) clearly shows that honeybees may be important pollinators of both native and alien plant species. On the other hand, in Brazil, honeybees have become important pollinators of the native *Dinizia excels* (Fabaceae) in fragmented habitats as they resulted in more than three times the seeds when compared to native bees in continuous habitats, and they have replaced these native bees (Dohzono and Yokoyama, 2010). Honeybees may indeed be both quantitatively effective or qualitative poorer pollinators in various systems (Traveset and Richardson, 2006). Thus, due to these equally plausible outcomes, much debate has arose in the past, particularly in Australia, about the role that introduced social bees play in the reproductive ecology of native and introduced plants and fitness of native biota (Gross and Mackay, 1998, Goulson et al., 2002, Hanley and Goulson, 2003).

Honeybees: their importance as pollinators of invasive plant species in southern Africa

The spread of invasive alien plant species from their sites of introductions in new biogeographical ranges may be facilitated by a number of factors, particularly by the establishment of positive interactions with the prevailing organisms in the new range (Richardson et al., 2000). The above is highlighted by invasive alien plant species integrating successfully within pollination webs of new ranges (Ford et al., 1979, Hanley and Goulson, 2003, Geerts and Pauw, 2009, Le Roux et al., 2010). Indeed, the chances of an introduced plant species becoming invasive may depend on how well it integrates with the prevailing pollination webs in the new system (Stout et al., 2006, Geerts and Pauw, 2009). Many introduced plant species typically exhibit generalized pollination syndromes with flowers having readily accessible rewards (Stout et al., 2006, Coombs and Peter, 2010). Indeed, introduced flowering plants exhibiting traits that allow them to overcome pollinator limitations in new ranges may become more invasive than plant species without these features (van

Kleunen et al., 2007a). One trait that may assist introduced plant species in new ranges to overcome pollinator limitation is a showy display of unspecialized flowers (Stout et al., 2006). Introduced plant species that have distinct showy unspecialized flowers are most likely to be attractive to native pollinators (Gibson et al., 2013) such as honeybees which are considered excellent generalist pollinators (Allsopp et al., 2008). For example, in Ireland where *Rhododendron ponticum* is invasive, its large floral display allows it to attract a variety of insects although pollination is mostly achieved by *Bombus* species and not by honeybees (Stout et al., 2006). In southern Africa, many Australian acacias have been introduced in the 19th and 20th century for a variety of reasons (Zenni et al., 2009), and these acacias are characterized by presenting dense clusters of flowers to attract unsuspecting and/or potential flower visitors. One *Acacia* species, *Acacia saligna*, introduced into southern Africa in the mid-19th century, has yellow inflorescences with numerous flowers that have rewards readily accessible to a wide range of flower visitors, thus making it more attractive (Gibson et al., 2012). *Acacia saligna* (Fabaceae) invades the fynbos vegetation of South Africa and is considered a problematic invasive plant species that transforms land and vegetation. In southern Africa, *A. saligna* has significantly usurped the native bees (*Apis mellifera* subspecies *capensis*) away from the native species *Roepera fulva* (Zygophyllaceae) which is florally similar and overlaps anthesis with the former (Gibson et al., 2012), and the native honeybees are suggested as potential cross-pollinators of this *Acacia* (Gibson et al., 2013). *Apis mellifera* subspecies *capensis*, native to the Cape region of southern Africa, have also been observed in Table Mountain Park pollinating the invasive Australian *Acacia paradoxa* (Zenni et al., 2009). Apart from the acacias, *Datura stramonium* (Solanaceae) is one other introduced plant species that might be potentially be pollinated by honeybees in its invasive range South Africa. *Datura stramonium* is an annual plant that has been introduced into southern Africa for more than 100 years, and today it occurs mainly in disturbed sites, agricultural and industrial sites, and in road sides (van Kleunen et al., 2007a). In South Africa, honeybees (possibly *A. mellifera*) were the main visitors to *D. stramonium* flowers and they actively collected pollen on flowers; accordingly, the authors suggested that honeybees might induce some cross-pollination to the latter plant and that they are also likely to induce self-pollination during pollen removal from anthers (van Kleunen et al., 2007a). In another study, native honeybees *Apis mellifera* were demonstrated to successfully pollinate the invasive "moth catcher" *Araujia sericifera* Brot. (Apocynaceae-Asclepiadoidea) (Coombs and Peter, 2010). *Araujia sericifera* is originally from South America but has invaded many countries including southern Africa where it grows in disturbed sites and in urban areas along fences. The moth catcher has white, dense, scent producing flowers throughout the day and night which were shown to be mainly visited and pollinated by honeybees during the day, with moths only making a negligible contribution to fitness of this plant species (Coombs and Peter, 2010). According to the above demonstrations, honeybees, although native

in southern Africa (Donaldson, 2002), play a crucial role in the reproductive ecology of introduced plant species and this is particularly important due to that these positive interactions may facilitate the naturalization (van Kleunen et al., 2007b) and hence invasiveness of these undesired plant species. It should also be borne in mind that the numbers of honeybees in habitats in southern Africa may be artificially high on account of the large number of managed hives.

Hummingbirds and passerine birds: their importance as pollinators of native & invasive alien plant species

Birds, as evidenced by several studies, are also flower visitors, nectar feeders, and important in providing pollination to native and, to a lesser extent, exotic plant species that occur in native and alien geographical ranges respectively (Ford et al., 1979, Botes et al., 2009, Geerts and Pauw, 2009, Glinos and Cocucci, 2011, Maruyama et al., 2015). The most important avian pollinator group in the Old World regions encompasses nectarivorous birds in the order Passeriformes (Ford et al., 1979, Geerts and Pauw, 2009) whereas in the New World, the important nectarivorous bird pollinator group is Trochilidae (Maruyama et al., 2015). The Old World nectarivorous passerines are the African and Paleotropical sunbirds within the family Nectarinidae (in Australia there is one species of sunbirds within the genus Nectarinia Ford et al., 1979) and the Australian honeyeaters, Meliphagidae, in Australasia (Ford et al., 1979, Geerts and Pauw, 2009). All passerines are characterized by short wings, comparatively larger body sizes (e.g., malachite males weight about 19.4 g), curved bills, and large feet, and their behavior when feeding on nectar in flowers displays rare occasions of hovering but they usually prefer perching (Geerts and Pauw, 2009). The New World Trochilidae are, in comparison to passerines, characterized by long, pointed wings; small, light bodies; long, straight bills (e.g., hermit hummingbird, bill length of approx. 2.8 cm Maruyama et al., 2015); small feet; and a tendency to hover when feeding off nectar in plants (Geerts and Pauw, 2009).

Plants that conform to bird pollination are characterized by a suite of traits that include copious volumes of dilute nectar, red to orange coloration, tubular perianths with no landing platforms, unscented flowers, and anthers with stigmas distant from the nectary (Ford et al., 1979, Westerkamp and Gottsberger, 2000, Johnson and Nicolson, 2008, Botes et al., 2009, Geerts and Pauw, 2009, Faegri and Van der Pijl, 2013). These features of bird pollinated plant species have been suggested to act as filters of inefficient animal pollinators that visit these flowers (Johnson et al., 2006). For example, it has been said that the red coloration in flowers filters insects visitors, and, nectar is thus

less likely to be depleted by these robbers as the red coloration is inconspicuous to most insect species (Ford et al., 1979, Johnson et al., 2006). Furthermore, other studies (in Johnson et al., 2006) have suggested that nectar may contain secondary compounds such as phenolics and alkaloids in order to filter out ineffective floral visitors. Pollination by birds may be more advantageous than pollination by insects in several regards. Birds, although requiring large amounts of nectar than insects, may increase the chances of outcrossing as they visit more flowers and fly further during feeding bouts (Ford et al., 1979, Ducroquet and Hickel, 1996). Although some insects (e.g., bumblebees) have longer daily activity periods, particularly in cool weather conditions where they can maintain body temperatures that are significantly higher than the surrounding environment and thus are able to forage very early or very late in the day in relation to other insects (McQuillan and Hingston, 1999, Goulson et al., 2002), birds in very cool weather conditions or in winter can be rendered reliable pollinators (Ford et al., 1979, Dohzono and Yokoyama, 2010). Bees often feed on nectar in blossoms that are considered "ornithophilous" (Johnson et al., 2006, Botes et al., 2009). However, other studies suggest that honeybees may be ineffective as pollinators of apparently bird-adapted plants (Botes et al., 2009, Maruyama et al., 2015) to an extent that they may reduce fitness of these "ornithophilous" plants (e.g., Dohzono and Yokoyama, 2010, Hargreaves et al., 2010).

Birds can be pollinators of exotic plant species, as evidenced by studies of the invasive *Nicotiana glauca* in southern Africa (Geerts and Pauw, 2009). *Nicotiana glauca* is native to northern Argentina where it is predominantly pollinated by hummingbirds. However, in southern Africa, *N. glauca* has integrated into the Old World pollination web that lacks hummingbirds and, instead, its flowers are pollinated by Malachite sunbirds (*Nectarinia famosa*) which successfully transfer pollen between plants and enhance seed set when compared to pollinator excluded flowers (Geerts and Pauw, 2009). Interestingly these perching birds often hover like hummingbirds when feeding on the flowers. In addition, it has been reported that the generalist and opportunistic behavior of honeyeaters in Australia allows them to feed on, and probably pollinate exotic plant species (Ford et al., 1979). From the above examples, particularly examples of invasive alien species, it becomes apparent that new or established mutualisms between one organism (either alien or indigenous organism) and the other (native or themselves introduced) very often enhance, and are important for, many invasion events or the success of native or introduced species (Richardson et al., 2000).

The importance of hybridization in invasion events

The spread of invasive alien plants in new biogeographical ranges may also be influenced by the potential to hybridize (Ward et al., 2012). Indeed, earlier records have demonstrated that naturalization, possibly followed by invasion, may be affected by the potential of species to hybridize with other species, and to introgress genes from other species (van Kleunen et al., 2007b). The potential to hybridize may also be particularly important for the evolution of breeding systems of invasive plants in that hybridization may allow opportunities to switch breeding systems of plants, as suggested that *Rubus alceifolius* in Madagascar and La Re'union which switched from being self-incompatible to being apomictic (van Kleunen et al., 2007c). In other cases, an invasive plant may simply switch breeding systems following introductions, as is the case with *Nicotiana glauca* (Solanaceae) that invades the Canary Islands and Greece where there are no specialized flower visitors such as hummingbirds and sunbirds, and this plant maintains abundant seeds through selfing in these invasive ranges (Ollerton et al., 2012). However, the hybridization with, and introgression of genes from, other species, and ultimately the integrity of closely related species may depend on processes which prevent inter-specific pollination such as mechanical and ethological isolation (Ellis and Johnson, 1999). If this is the case, visits of pollinators to closely related species, or distantly related species may not be beneficial since this would waste pollen and ovules if post-pollination events results in inviable seeds or unfit offspring due to genetic incompabilities (Ward et al., 2012). Nevertheless, it has been shown that non-native species often hybridize with other species, which are either native or themselves exotic, and this has resulted in the origin of new forms of sexually reproducing plant species and most of these "new" forms (even new species in the case of allopolyploids) have spread beyond their sites of introduction (Ellstrand and Schierenbeck, 2006). Ultimately, these new lineages with a long history of hybridization may be genetically advantageous when compared to their parental plants as they may be relatively genetically diverse (Schierenbeck and Ellstrand, 2009).

Hypotheses and aims

In this study, I explore the breeding systems, pollination biology, and hybridization potential of *Canna indica* L. and two forms of *Canna glauca* L., which are all naturalized in South Africa. The motivation behind this study is that the reproductive biology of introduced *Canna* is not well understood, apart from some preliminary data existing for *Canna indica* L. (Rambuda and Johnson, 2004). In fact, only two studies document the breeding systems and pollination biology of *Canna* in their native range (Glinos and Cocucci, 2011, Maruyama et al., 2015). Ultimately, this leaves the scientific community with little information about the pollination or breeding system biology of

Canna. The most influential motivation driving this study is that some species of *Canna* are invasive, including in southern Africa (Rambuda and Johnson, 2004, Maas-van de Kamer and Maas, 2008, Prince, 2010, Wilson et al., 2013). Finding out about the reproductive traits and pollination biology *Canna* can be valuable because such information could contribute to the effective control of these species. For example, nurseries could be prohibited from selling *Canna* species that are likely to become invasive in the future. In line with Baker's hypothesis, I hypothesize that the *Canna* species naturalized in South Africa are self-compatible and possibly autogamous, and that they are not pollen limited. I also hypothesize that the *Canna* species are pollinated by honeybees due to my preliminary observations which showed that bees are frequent visitors and that sunbirds appeared to rob nectar of flowers rather than contacting the anthers and stigmas. In addition, I hypothesize that the study species can hybridize freely on account of the plethora of hybrids in horticulture and the variety of mixed coloured plants occurring among the original species at my study sites.

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Chapter 2

Breeding systems and potential for hybridization in *Canna* species naturalized in South Africa

Abstract

A key aim of invasion biology is to identify characteristics of species that contribute to invasiveness. Breeding systems such as self-compatibility and autogamy as well as hybridization and vegetative reproduction are some aspects of reproductive biology that have been considered to play a role in the process of invasion by exotic plant species. In this study, the reproductive biology of two *Canna* species (*C. indica* and *C. glauca*) was assessed in order to better understand the risks associated with their invasiveness in southern Africa. Using controlled experiments, I established that *C. indica* is largely autogamous and fully self-compatible, whereas orange- (OF) and yellow-flowered (YF) forms of *C. glauca* are allogamous (e.g., dependent on pollinators) and partially and fully self-compatible, respectively. Unexpectedly, given that habitual selfers should purge genetic load, selfed progeny of *C. indica* showed evidence of inbreeding depression, whereas selfed progeny of *C. glauca* OF did not show inbreeding depression. I also found that both *C. indica* and *C. glauca* OF are inter-fertile with *C. glauca* YF. None of the study taxa are apomictic, but all can reproduce vegetatively. These results show that both of the *Canna* study species are capable of uniparental reproduction. Autogamy in *C. indica* is significant as this species has been reported to be allogamous in its native range. Hybridization could introduce new genotypes into populations and populations could expand through vegetative reproduction. *Canna* species thus pose a significant risk, particularly because their favoured habitat is sensitive wetland vegetation.

Key words*: invasive alien plant species;* Canna*; Baker's Rule; hybrids; inbreeding depression*

Introduction

Recent reports estimate that approximately 33 000 plant species have been introduced in areas where they are not native (Latombe et al., 2017). Many of these introduced plant species reproduce efficiently following introductions (Bufford and Daehler, 2014) and spread from sites of

introductions to present a variety of effects to the functioning of surrounding ecosystems (Richardson et al., 2000). Despite the staggering number of introduced plants in new ranges, only a small proportion of introduced plant species has become invasive (Ellstrand and Schierenbeck, 2006, Stout et al., 2006, van Kleunen and Johnson, 2007a). However, given the current number of species occurring in new ranges and their potential negative effects, it is an urgent key aim of invasion biology to formulate generalized models and identify features that promote invasiveness of species (Stout et al., 2006, Hao et al., 2011).

Assessment of the reproductive biology of species is still highly researched and has contributed to development of accurate screening protocols (e.g., Rambuda and Johnson, 2004, van Kleunen and Johnson, 2007b, van Kleunen et al., 2007b, Gibson et al., 2011, Bufford and Daehler, 2014, Bufford et al., 2016). Specifically, assessing breeding systems of naturalized and/or invasive plant species is important because these breeding systems may affect the dynamics of targeted populations by supplying propagules via seeds, of varied quality and quantity (Ward et al., 2012, Bufford and Daehler, 2014). Knowledge of breeding systems of naturalized plant species can help inform risk assessments as well as estimations of hybridization potential (Ugoletti et al., 2013), and studies involving these assessments have been proposed for inclusion in management programs (Le Roux et al., 2010).

Studies of the breeding systems of invasive alien plant species are based largely on theory developed by Herbert Baker in the 1950s (van Kleunen et al., 2007c, Harmon-Threatt et al., 2009). Baker's hypothesis posits that self-compatible species, especially those capable of autonomous self-pollination (or self-fertilization), are most likely to be invasive or to be the dominating colonizers in a new range where suitable pollinators and/or mates are limited following long-distance dispersal (Baker, 1967, Pannell and Barrett, 1998). Baker's hypothesis is highly relevant to invasive species because the new ranges in which these species are introduced are generally expected to be deprived of suitable mates and/or pollinators (Brys and Jacquemyn, 2011). The lack of suitable pollinators means that allogamous alien species are most likely to experience reduced seed set due to deposition of an insufficient number of pollen grains, which may also be of low quality (Parker, 1997, Brys and Jacquemyn, 2011). As a consequence, the spread of aliens that do not have capacity for uniparental reproduction, and autonomous selfing in particular, is likely to be limited (Le Roux et al., 2010). Questions have been raised about the generality of Baker's hypothesis. The first of these was from the botanist Sherwin Carlquist in 1966 noting the wide occurrence of outcrossing in island floras (Baker, 1967), many of which have a high proportion of dioecious taxa (Bawa and Beach, 1983, van Kleunen and Johnson, 2007a, van Kleunen et al., 2007c, Pannell, 2015). Despite

these doubts, Baker's hypothesis may be applicable in some cases, such as when a population has to be established following introductions and when initial expansion is needed to occupy new available habitats (Pannell, 2015). Species introduced in abundance by humans or natural dispersal agents are less likely to require uniparental reproduction at later stages (Pannell et al., 2015b). Explicit tests of Baker's hypothesis provide results that support Baker's hypothesis (Rambuda and Johnson, 2004, van Kleunen et al., 2007b). Given the small number of such studies, there is a need for additional empirical studies to support or discredit Baker's hypothesis in this context.

A general pattern among invasive alien species is that they have high reproductive outputs despite the lack of suitable mates and/or pollinators in new ranges (e.g., Hao et al., 2011, Moore et al., 2011, Kaplan et al., 2014). Self-compatibility may likely provide an additional source of pollen to fertilize more ovules of the same individual (Barrett and Harder, 1996, Barrett, 2002) to ultimately counteract pollen limitation caused by a deposition of an insufficient number of pollen grains in cases where aliens are not fully adapted to pollinators or when pollinator visits to populations occur rarely due to Allee effects (Lamont et al., 1993, Stephens et al., 1999, Rambuda and Johnson, 2004, van Kleunen et al., 2007a). In addition, autonomous self-fertilization may alleviate both mate and/or pollinator limitation even further because species would rarely require the services of pollinators (Barrett and Harder, 1996) or mates to set seeds. Therefore, capacity for uniparental reproduction and autonomous selfing in particular may facilitate the naturalization and ultimately the invasiveness of introduced alien species (Rambuda and Johnson, 2004, van Kleunen and Johnson, 2007b).

Problems associated with selfing include inbreeding depression, which is the reduction of fitness of selfed derived offspring in comparison to outbred derived progeny (Eppley and Pannell, 2009). Inbreeding depression may be particularly more prevalent in small populations in part because related individuals within are more likely to mate with each other (Zhou and Pannell, 2010). However, studies have shown that species that have inbred for a long time usually purge deleterious recessive alleles and are thus less likely to show increased inbreeding depression in comparison to species that have historically experienced high rates of outcrossing (Barrett and Harder, 1996). It is therefore important to further investigate the levels of inbreeding depression in invasive species (Ward et al., 2012).

Vegetative reproduction may be an additional aspect of plant life histories that promotes invasiveness (Gibson et al., 2011). Vegetative reproduction can strongly promote increases in the size of existing populations, but may not contribute as much as seeds to long-distance dispersal and

thus establishment of new populations (van Kleunen and Johnson, 2007a). Vegetative reproduction may be particularly important for species with minimal or no ability for seed set via autogamous reproduction or co-option of existing pollinators (Pannell and Barrett, 1998).

Another component that is particularly important for plant invasions is the potential of species to hybridize (Ward et al., 2012, Ugoletti et al., 2013). Hybridization can result in hybrid vigour through heterosis and can accelerate the development of variation that makes colonization of new environments possible (Ellstrand and Schierenbeck, 2006, Ugoletti et al., 2013). Hybridization may even allow for the evolution of breeding system characteristics in plants (e.g., plants switching from a self-incompatible to a self-compatible breeding system, van Kleunen et al., 2007c, Schierenbeck and Ellstrand, 2009). Hybridization among invasive plants has been documented previously (e.g., milkweed species, Apocynaceae, Asclepiadoideae), and the hybridization process may involve combinations of invasive and non-invasive alien species, or even alien and native species (Ward et al., 2012). Current knowledge about the potential for alien species to hybridize is limited and often missing in legislation (see example list of invasive grass hybrids not listed in NEM: BA regulations Visser et al., 2017). This information may be particularly important for the development of informed and updated risk assessment protocols. South Africa often uses the Australian Weed Risk Assessment Protocol, which incorporates hybridization potential of species (e.g., Zenni et al., 2009, Geerts et al., 2013, Kaplan et al., 2014, Nxumalo et al., 2016); thus inclusion of studies of hybridization potential of species may be invaluable for risk assessment protocols.

In this study, I assess and document the breeding systems, hybridization potential, and vegetative reproduction in *Canna indica* L. and two colour forms of *Canna glauca* L. which are naturalized in South Africa. Although there are some assessments of breeding system characteristics of *Canna* species in their native ranges (Glinos and Cocucci, 2011, Maruyama et al., 2015), and other Zingiberales (Wang et al., 2005, Ma et al., 2012), relatively little information is available about the reproductive biology of *Canna* in introduced ranges (Rambuda and Johnson, 2004) where most available information is anecdotal (Maas-van de Kamer and Maas, 2008). The motivation behind this study is that some species of *Canna* are invasive in some parts of the world, including southern Africa (Rambuda and Johnson, 2004, Maas-van de Kamer and Maas, 2008, Prince, 2010), and the overall risk of invasiveness needs to be assessed. *Canna indica* L. is a declared category 1 b and/or category 1 invasive plant in South Africa according to the NEM: BA and the CARA Regulations respectively (DoEA, 2009, Wilson et al., 2013). The study taxa often occur sympatrically, thus increasing the risk of on-going hybridization. I hypothesized that *Canna* study taxa are self-compatible and possibly autogamous on account of the close proximity of pollen and stigma on the

pollen presenter (Maas-van de Kamer and Maas, 2008, Glinos and Cocucci, 2011). I also hypothesized that the study taxa will have high levels of inter-fertility, given the large number of hybrids available in horticulture (Gupta et al., 2013). Finally, I sought to examine the extent of vegetative reproduction and inbreeding depression in populations of the taxa.

2. Methods

2.1. Study species or taxa

Canna in the order Zingiberales (Baran et al., 2010, Huber et al., 2013) is the only genus within the family Cannaceae (Prince, 2010). It is comprised of herbaceous species with annually renewable shoots that live for a few months following flowering (Maas-van de Kamer and Maas, 2008), and the clump of progressive shoots may live for several years (Soares et al., 2005, Gupta et al., 2013). The genus comprises approximately 10 - 20 species (Kress and Specht, 2006, Mishra et al., 2013) and a staggering 1000 plus hybrids (Prince, 2010, Gupta et al., 2013). *Canna* species originate in the Americas (Soares et al., 2005, Baran et al., 2010), but today they are widely distributed throughout the tropical and subtropical regions of the world (Prince, 2010, Tobar-Vargas et al., 2013) owing to their extensive use as ornamentals by the horticultural trade industries (Young, 1982, Baran et al., 2010).

Canna indica L. is native to South America and in North America up to Mexico (Glinos and Cocucci, 2011). *Canna indica* is characterized by its habitat preference in wet forests, along rivers or road sides in secondary vegetation (Maas-van de Kamer and Maas, 2008). The genus has flowers with petals that are fused at the base and petals are further fused with the stamen and 3 staminoids (Glinos and Cocucci, 2011). The overall flower colour of *C. indica* is red or orange and with individuals that can grow up to 2 m. The distinctive feature of this species is that the lower sides of leaves are glabrous (Maas-van de Kamer and Maas, 2008). *Canna glauca* L. is native to South America as far south as northern Argentina (Maas-van de Kamer and Maas, 2008). In this study, two colour forms of *C. glauca* were studied; one with orange flowers (e.g., *C. glauca* OF) while the other form had yellow flowers (*C. glauca* YF). *Canna glauca* grows in marshes, swamps, and along margins of lakes (Armbruster et al., 1999, Maas-van de Kamer and Maas, 2008). Individuals of this species may reach up to 3.5 m in height. *Canna glauca* usually has leaves that are dull grey, green, or bluish in colour (Maas-van de Kamer and Maas, 2008).

All species of *Canna* exhibit secondary pollen presentation (Howell et al., 1993, Maruyama et al., 2015) where shortly before anthesis, pollen from the anther is deposited in a clump directly underneath the receptive part of the stigma (Glinos and Cocucci, 2011). Secondary pollen presentation has been said to facilitate accurate pollen transmission (Howell et al., 1993). *Canna indica* in its native range Argentina is efficiently pollinated by a hummingbird, *Heliomaster furcifer* (Glinos and Cocucci, 2011). In addition, in Argentina, this species is reported to be allogamous (Glinos and Cocucci, 2011) but in its introduced range in southern Africa it has been reported to be fully autogamous (Rambuda and Johnson, 2004). *Canna glauca* in its native range has been reported to be visited and possibly pollinated by hummingbirds, butterflies, honeybees and moths (Armbruster et al., 1999, Aizen et al., 2016) and is presumed to be self-compatible (Maas-van de Kamer and Maas, 2008).

2.2. Study Sites

Field studies were conducted in 2014 (February to October), 2015 (February to October), 2016 (October) and 2017 (June). Field experiments were conducted at one site in Pietermaritzburg, and at one site in Howick, South Africa. The site in Pietermaritzburg (S 29°38'9.002" E 30°25'5.998") has populations of both forms of *C. glauca* that occur sympatrically with *C. indica* in a wetland in savanna vegetation. The site in Howick (S 29°28'50.999" E 30°13'52.003") has populations of both *C. glauca* colour forms in a wetland in grassland vegetation.

2.3. Breeding system assessment

To assess the capacity for uniparental reproduction and autonomous self-fertilization, controlled pollination experiments using flowers bagged prior to anthesis were conducted on the study plants.

In 2014 and 2015, only *C. indica* and *C. glauca* OF received all the treatments. Whole plants (60 and 40 plants for each year, respectively) were assigned to the following treatments; (1) emasculation to test for apomixis; (2) bagged and unmanipulated to test for autonomous seed production; (3) self-pollination using pollen from the same test plants to test for self-compatibility; and (4) cross-pollination using donors that were at least 10 m away from the test plants and usually in a different patch as a positive control.

In 2017, *C. indica* and both colour forms of *C. glauca* received all the treatments. In this case, however, a split-plot design was used, where four flowers were used on each of 15 plants per form and each flower on a plant was assigned to a different breeding system treatment.

In all years, after four weeks, fruits from all treatments were harvested and the proportion of flowers setting fruits was calculated. For each fruit, the number of viable and aborted seeds as well as number of unfertilized ovules were counted on a dissecting microscope (A. Krüss Optronic – Germany; MSZ5000-T-IL-TL Stereo Microscope). Lastly, seeds from each fruit were weighed on an electronic balance and their weights recorded.

2.4. Hybridization

To assess inter-fertility among study species and forms, a set of controlled hybridization treatments were carried out in 2017 where four flowers were used on each of 15 plants, and each flower on a plant was assigned to a different hybridization treatment. Flower buds were covered with mesh bags and after anthesis four flowers on each plant were each assigned to the following treatments; (1) self-pollination (with pollen from same plant); (2) cross-pollination (with pollen from a conspecific plant that is at least 10 m away and from a different patch), and (3) and (4) interspecific crosses with one of the other forms.

2.5. Inbreeding depression assessment

Seeds that were used for germination tests and for investigating the extent of inbreeding depression in *C. indica* and *C. glauca* OF were obtained from the 2015 breeding system treatments (98 seeds from selfing and 201 seeds from outcrossing for *C. indica* and 204 seeds from selfing and 181 seeds from outcrossing for *C. glauca*). All seeds were mechanically scarified with a metal file and later sowed in six-hole seedling trays. Each hole had four seeds, and each tray had seeds of the same species and same treatment. Trays were placed in a green house in a stratified manner, and seeds were watered every day from September 2016 following sowing. Germination success of seeds was measured after seven, and 14 days. With maintained watering, measurements of seedling heights, heights of longest leaves, widths of leaves, and number of leaves in seedlings were recorded after 28, and 56 days of germination.

2.6. Vegetative reproduction assessment

To assess whether plants have the capacity to reproduce vegetatively, five plants for each species/form were dug out from the soil to check root connections between neighbouring plants.

2.7. Data Analysis

Breeding system data from 2014 and 2015 (hereafter 2014/15) were combined for analysis, and that of 2017 was analysed separately. This was due to a change in the experimental design where a single treatment was applied to each plant in 2014/15, while a split-plot design (several treatments per plant) was used in 2017. Data for masses of seeds only includes the years 2015 and 2017, and these data are analysed separately for each year for each species/plant form. All analyses were done using Generalized Estimating Equations (GEE) to deal with potential non-independence of data, except the analyses of seed masses in 2015, which was done using simple Generalized Linear Models (GLM) as plant identity was not available for each seed (seeds from a treatment were pooled). The software used was IBMs SPSS® Statistics, version 23/24. In the GEEs used to analyse most data, the subject variable in 2014/15 breeding systems was the year; in the 2016 seed germination data the subject was tray identity, and in the 2017 breeding systems data, and in hybrids data the subject variable was plant identity. All proportions (i.e., of flowers setting fruits, aborted ovules, ovules setting seeds, and percentage germination) were analysed using a model, where the probability distribution was binomial and the link function was logit. The predictors were controlled pollination treatments and species/plant form, the events variables were the no. of fruits, aborted ovules, no. of seeds, no. of germinated seeds, and the trial variables were the no. of flowers, no. of all ovules (e.g., all unfertilized and aborted ovules plus no. of seeds), and no. of sowed seeds in a tray. All the measured plant characteristics in germination tests (except percentage germination) were analysed using a normal distribution and identity link. Only germination success of day 14 and measured plant characteristics at day 56 were used for analysis. For GLM, the likelihood ratio chi-square was used as a statistic to test the model effects (set at type III). For GEE, significance was assessed using Wald or Score statistics depending on sample sizes (the former was used for small sample sizes). Significant differences (at $p < 0.05$) between treatments in the pairwise comparisons were based on the Sequential Sidak adjustment.

All masses of seeds in 2015 (GLM) and 2017 (GEE) were analysed using the Identity Link function, where the probability distribution was normal. The factor was the treatment; the dependent variable was the mass of seeds per fruit.

In all taxa across all years, indices for self-compatibility were calculated as s/c, where s = the mean number of seeds per flower for self-pollination and c = the mean number of seeds per flower for cross-pollination. Indices were also calculated for autonomous self-pollination as ratios of mean number of seeds per flower in the bagged control treatment over the mean number of seeds per flower for self-pollination (Rodger et al., 2010).

Plants were considered inter-fertile when fruit and seed set of hybrids did not differ significantly among the conspecific and interspecific crosses.

3. Results

3.1. Breeding system assessments

3.1.1. Canna indica

In 2014/15, the breeding system treatments had no significant effect on fruit set (Appendix, Fig. 1 A). In 2017, however, fruit set in the treatment to test for autogamy (hereafter autogamy treatment) was similar to that resulting from self-pollination, but lower than that resulting from cross-pollination; fruit set in self- was also similar to that of cross-pollination treatment (Fig. 1 A).

In 2014/15, flowers in the autogamy treatment group had seed set similar to that of self-, but both the afore-mentioned treatments were different to the cross-pollination treatment which had a one-fold increase of seed set (Appendix, Fig. 1 B). In 2017, flowers in the autogamy treatment group had seed production similar to the self- but both autogamy and self- were lower than the cross-pollination treatment (Fig. 1 B).

In 2014/15, the breeding system treatments had no significant effect on ovules aborted (Appendix, Fig. 1 C). In 2017, flowers in the autogamy treatment group had aborted more ovules than those in both the self- and cross-pollination treatment groups which were similar (Fig. 1 C).

In 2015, the autogamy treatment produced seeds weighing less than those of both the self- and cross- treatments and the self- and cross-pollination treatments were different (Appendix, Fig. 1 D). In 2017, the autogamy treatment had produced seeds with similar weight to those resulting from the

self-treatment, but both these treatments had seeds weighing less than those resulting from the cross-pollination treatment (Fig. 1 D).

3.1.2. Canna glauca

During the years 2014/15, fruit set in the autogamy treatment for *C. glauca* OF was approximately five-fold less than the self- and cross-pollination treatments which had similar fruit sets (Appendix, Fig. 2 A). Similar results were obtained in the 2017 experiments involving both the orange and yellow colour forms of *C. glauca* (Fig. 2 A and Fig. 3 A respectively).

In 2014/15, the autogamy treatment in *C. glauca* OF led to the lowest seed production following the self- and then the cross-pollination treatments (Appendix, Fig. 2 B). In 2017 similar results were obtained for *C. glauca* OF (Fig. 2 B), with *C. glauca* YF having lower levels of seeds from the autogamy treatment and similar seed levels from the self- and cross-pollination treatments (Fig. 3 B).

Levels of ovule abortion were highest for selfed seeds of *C. glauca* (Fig. 3 C; Fig. 2 C).

In 2015, the breeding system treatments had no significant effect on masses of seeds of *C. glauca* OF (Appendix, Fig. 2 D). However, in 2017, the autogamy treatment had produced lighter seeds followed by the self- and the cross-pollination treatments (Fig. 2 D). The autogamy treatment of *C. glauca* YF in 2017 resulted in seeds weighing less than both the self- and cross-pollination treatments, of which both the afore-mentioned treatments had similar seed masses (Fig. 3 F).

In all species/plant forms across all the study years, no fruits were produced when flowers received the emasculation treatments used to test for non-pseudogamous apomixis.

Indices of autonomous self-pollination were 0.6 and 0.6 for *C. indica* in 2014/15 and 2017 respectively. For *C. glauca* OF in 2014/15 and 2017, autonomous self-pollination indices were 0.1 and 0.1 respectively. In 2017, index for autonomous self-pollination in *C. glauca* YF was 0.0. Indices for self-compatibility in *C. indica* were 0.7 and 0.5 in 2014/15 and 2017 respectively and indices were 0.8 and 0.5 for *C. glauca* OF in 2014/15 and 2017 respectively. Index for self-compatibility in *C. glauca* YF in 2017 was 0.7.
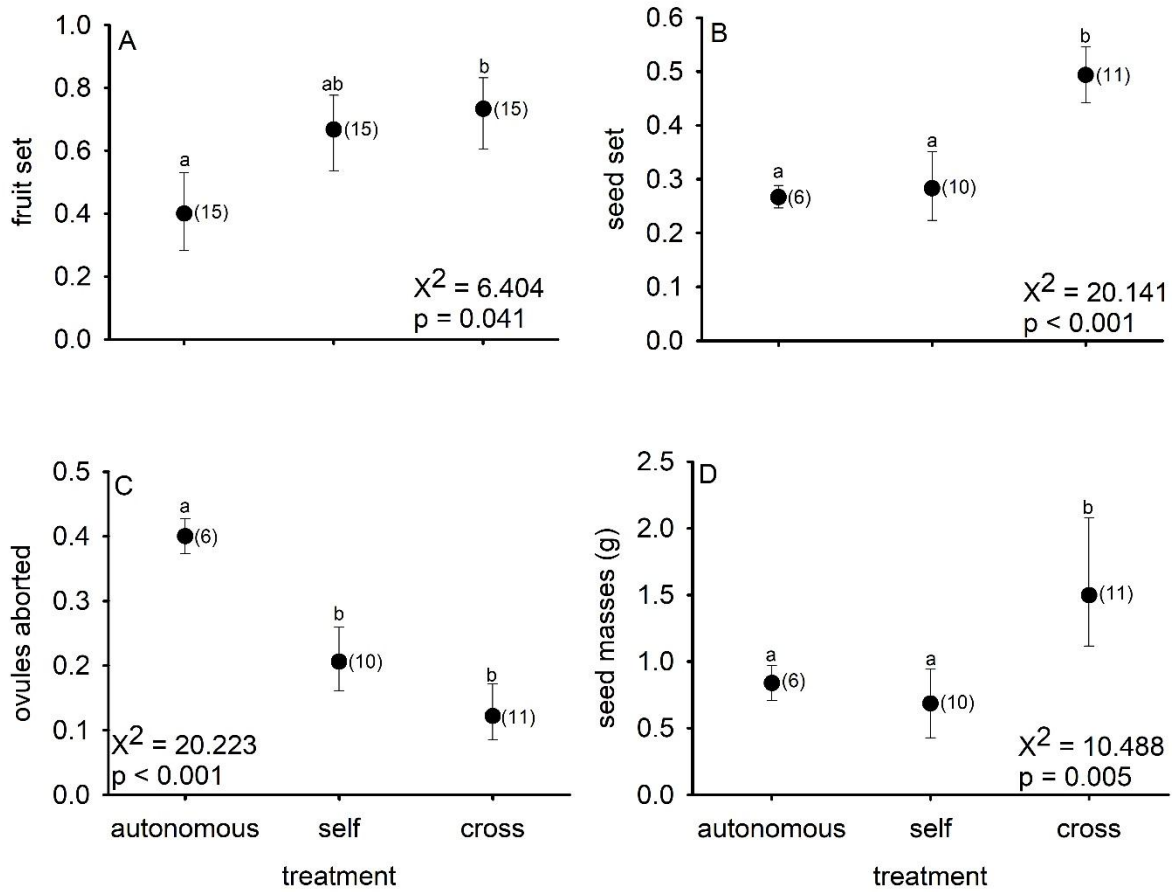
Figure 1: Mean values (± SE) of breeding system treatments of *C. indica* during 2017. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated. Means that share letters are not significantly different.
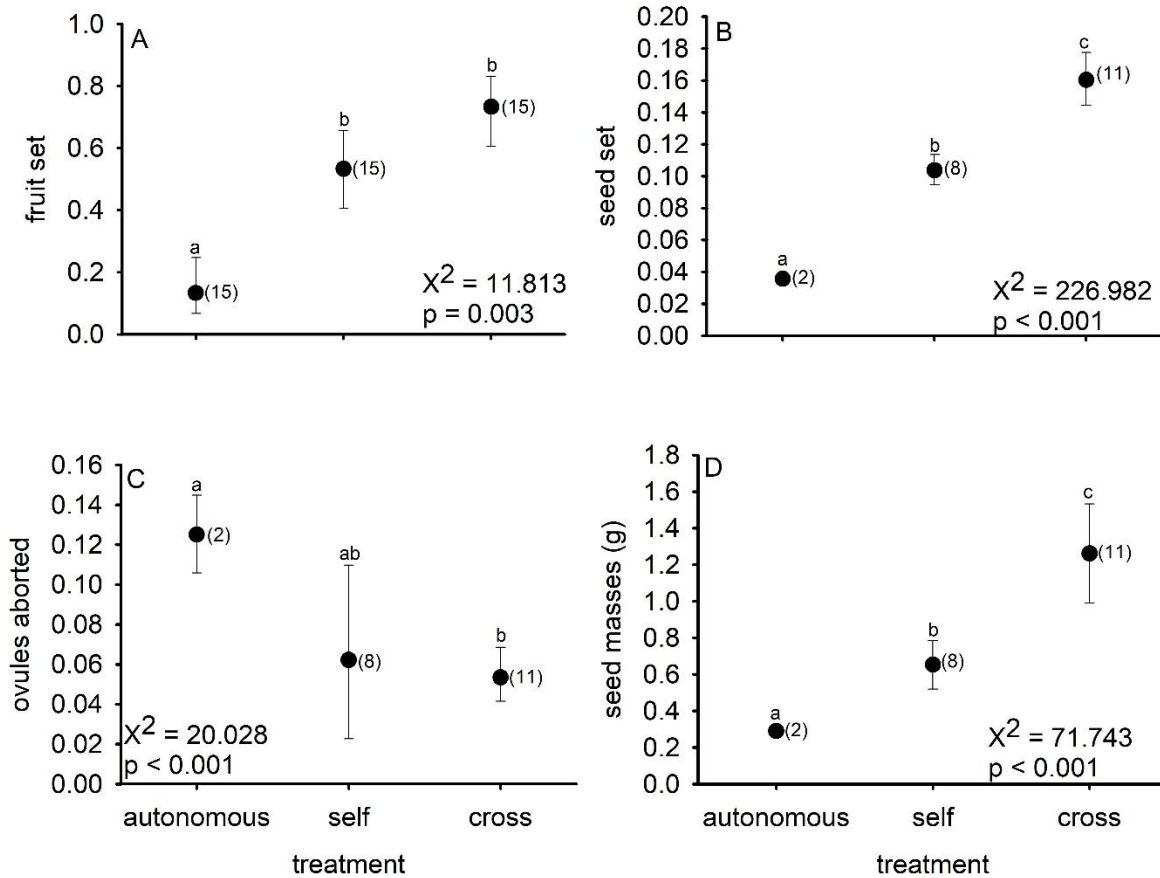
Figure 2: Mean values (± SE) of breeding system treatments of *C. glauca* OF during 2017. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated. Means that share letters are not significantly different.
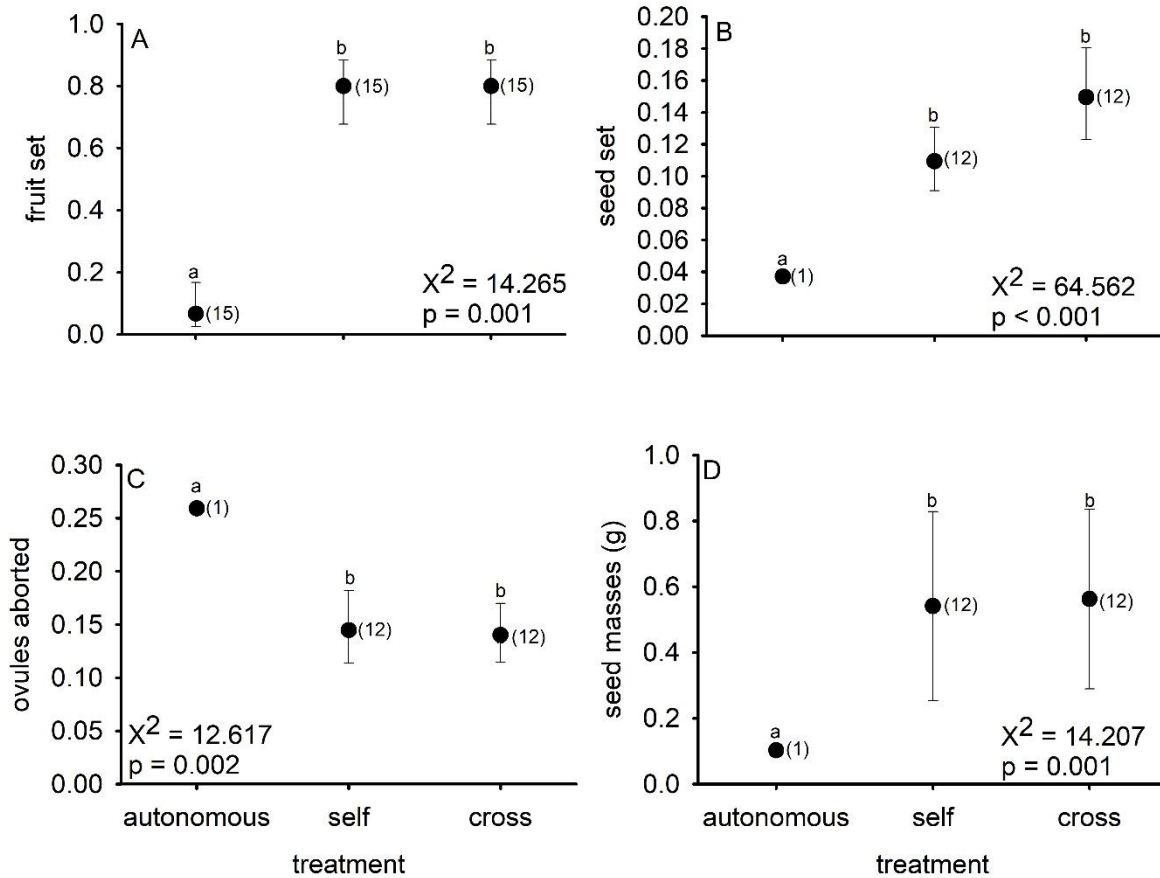
Figure 3: Mean values (± SE) of breeding system treatments of *C. glauca* YF during 2017. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated. Means that share letters are not significantly different.

3.2. Hybridization

Pollination with C. *indica* pollen resulted in similar fruit set levels for all three types of maternal parents (Fig. 4 A). Similar results were obtained when *C. glauca* YF pollen was used (Fig. 6 A). When *C. glauca* OF pollen was used as the sire, fruit set in the *C. glauca* OF maternal parent was higher than that for the *C. indica* maternal parent (Fig. 5 A).

In terms of seed set, *C. indica* × *C. indica* crosses produced significantly more seeds than other crosses (Fig. 4 B). Only *C. glauca* OF pollen had no significant effect on seed set of all maternal plants (Fig. 5 B). When *C. glauca* YF was used as the sire, a significant difference was found in seed set between the two maternal forms of *C. glauca* (Fig. 6 B).

Siring with *C. indica* had no significant effect on ovules aborted in all maternal plants (Fig. 4 C). In other crosses, seed abortion was lowest for the *C. glauca* OF maternal parent (Fig. 5 C, 6 C).

Crossing combinations did not affect the overall mass of seeds produced (Fig. 4 D, 5 D, and 6 D).
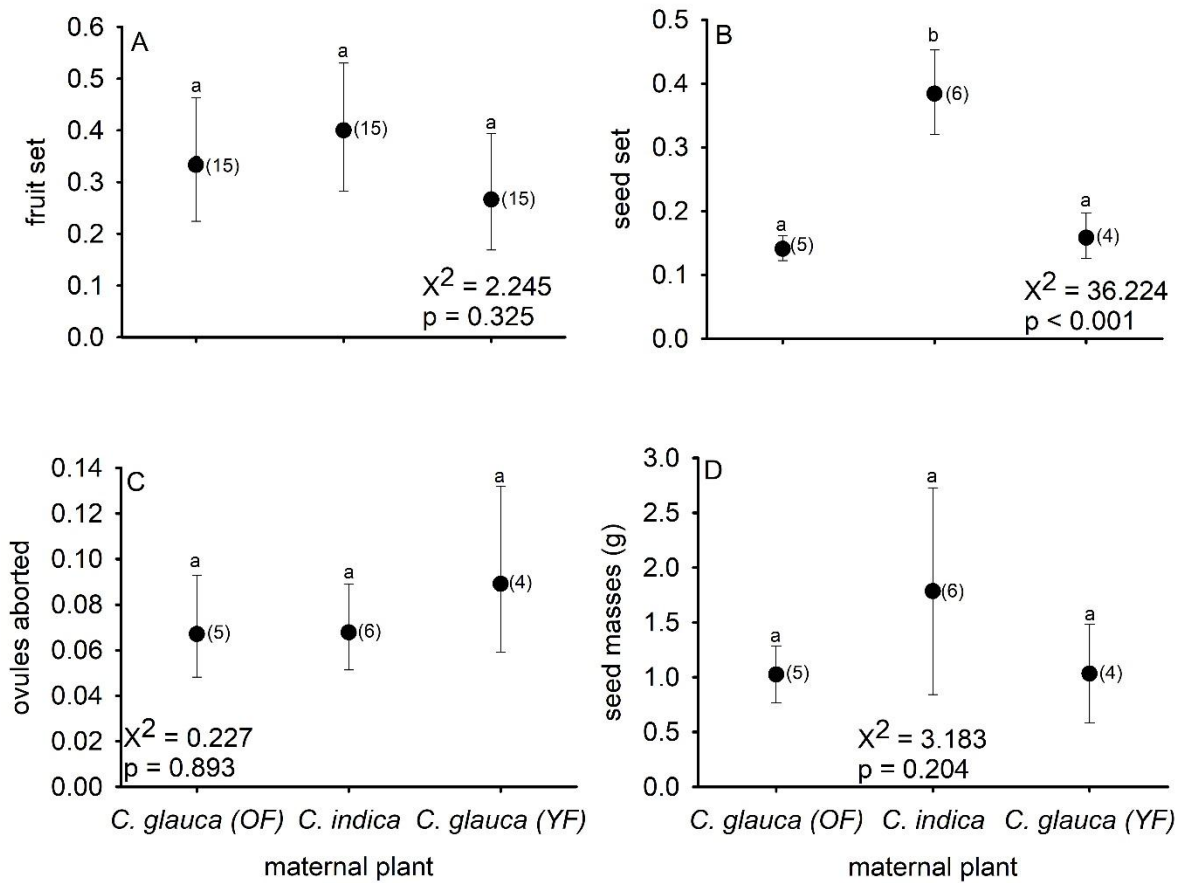

Figure 4: Mean values (± SE) of conspecific and interspecific-crosses of maternal *C. indica*, *C. glauca* OF, and *C. glauca* YF with paternal *C. indica* pollen to infer hybridization potential of the sympatric species. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in parentheses represent number of plants (with one flower) treated. Means that share letters are not significantly different.
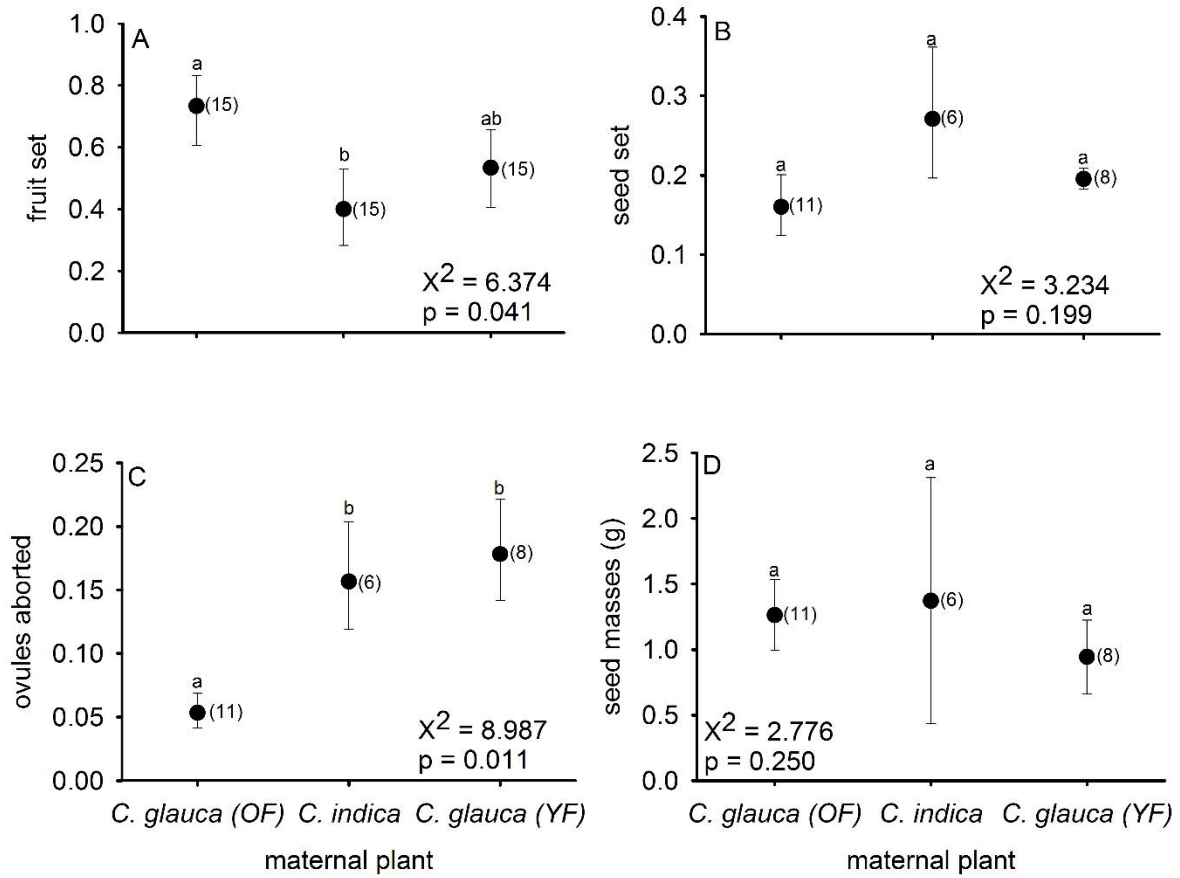
Figure 5: Mean values (± SE) of conspecific and interspecific-crosses of maternal *C. indica*, *C. glauca* OF, and *C. glauca* YF with paternal *C. indica* pollen to infer hybridization potential of the sympatric species. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in parentheses represent number of plants (with one flower) treated. Means that share letters are not significantly different.
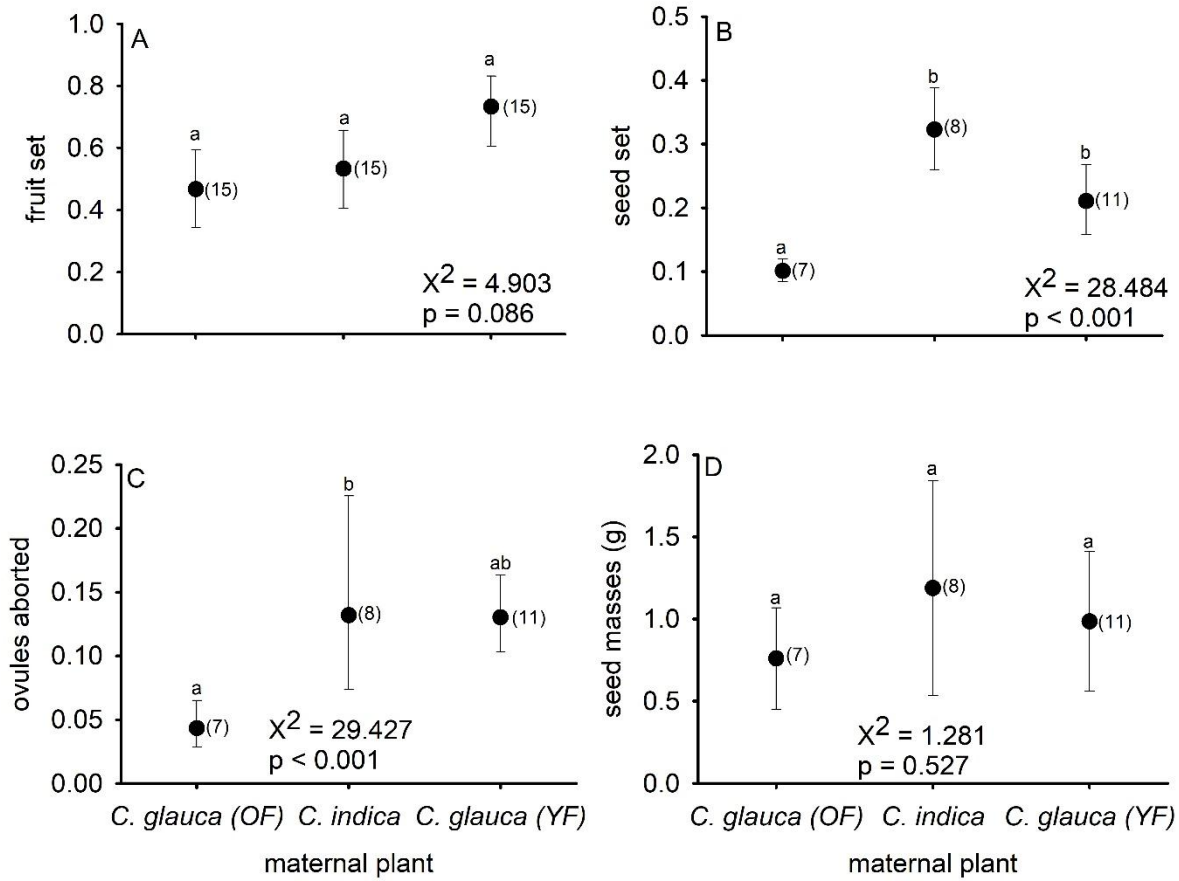
Figure 6: Mean values (± SE) of conspecific and interspecific-crosses of maternal *C. indica*, *C. glauca* OF, and *C. glauca* YF with paternal *C. indica* pollen to infer hybridization potential of the sympatric species. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated. Means that share letters are not significantly different.

3.3. Inbreeding depression assessment

In *C. indica*, all measured plant characteristics, except the number of leaves in seedlings (Fig. 7 E), were significantly different between the seeds and seedlings derived from the cross- and self-pollination treatments, with cross-pollination treatments outperforming the self-pollination treatments (Fig. 7 A, B, C, and D). In *C. glauca* OF, there were no significant differences registered between treatments for all measured parameters (Fig. 7 A – E).
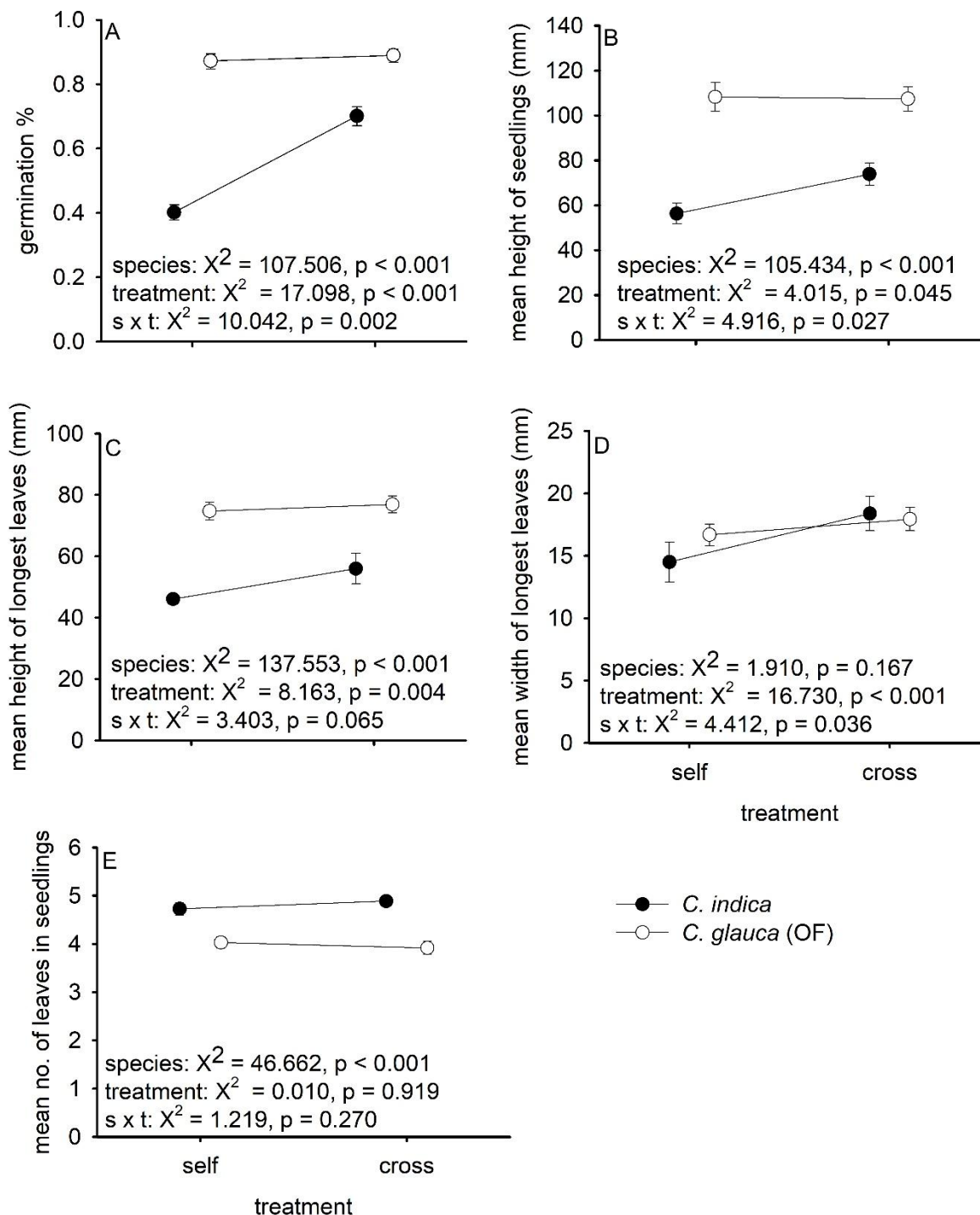
Figure 7: Germination success of seeds and performance of seedlings of *C. indica* and *C. glauca* OF across hand self- and hand cross-pollination treatments used to infer the extent of inbreeding depression in the studied species.

3.4. Vegetative reproduction assessment

When plants of each of the study species were dug out of the soil, they all had roots interconnected with neighbouring plants of the same species.

Discussion

The results of this study indicate that all study taxa are incapable of apomictic reproduction but are capable of sexual forms of uniparental reproduction. However, this capacity varies with each taxon in their introduced range, South Africa. Here, I have found that *C. indica* is self-compatible and is partially autogamous, e.g., able to set fruits mostly and seeds automatically without the services of pollinators (see Appendix, Fig. 1 A and B; Fig. 1 A and B). *Canna glauca* OF is partially self-compatible and is incapable of automatic self-pollination (see Appendix, Fig. 2 A and B; Fig. 2 A and B). Finally, *C. glauca* YF, is also incapable of automatic self-pollination; however, this form is fully self-compatible (Fig. 3 A and B).

In Argentina, *C. indica* has been found to be incapable of automatic self-pollination (Glinos and Cocucci, 2011). In contrast, I found that this species is partially capable of automatic self-pollination in its invasive range in South Africa (Appendix, Fig. 1 A and B; Fig. 1 A and B). A similar result was recorded by Rambuda and Johnson (2004). Therefore, the results of this study indicate a risk factor for invasiveness as per according to the NEM: BA regulations (DoEA, 2009). The other two studied *Canna* taxa (*C. glauca* YF and OF) are fully and partially self-compatible, respectively. However, neither is autogamous. *Canna paniculata* has been found to be self-compatible in Brazil (Maruyama et al., 2015). Therefore, self-compatibility seems to be common within this genus. However, more studies are required to verify this particularly in *Canna* species used in horticulture but not invasive.

The fact that *C. indica* in its native range has been shown to be allogamous (Glinos and Cocucci, 2011), while my study and that of Rambuda and Johnson (2004) have shown capacity for autogamous reproduction, may indicate that breeding systems have evolved in the invasive range of this species (van Kleunen et al., 2007c). Alternatively, it could mean that there is variation in the breeding system in the native range and that a selfing form was introduced to South Africa, or even that ability to self autogamously was derived by hybridization with other *Canna* species during the invasion process. Another study has shown that *Rubus alceifolius* (Rosaceae) switched from being self-incompatible in its native range in Vietnam to become apomictic in its introduced range in Mada-

gascar (Amsellem et al., 2001). Furthermore, the milkweed *Gomphocarpus physocarpus* (Apocynaceae, Asclepiadoideae) has switched from self-incompatibility in its native range to self-compatibility in Australia where it is declared invasive (Ward et al., 2012). The switch in breeding systems may be particularly correlated with the potential of a species to hybridize (van Kleunen et al., 2007c). Nonetheless, the current study's results, and those of Rambuda and Johnson (2004) show increased support for Baker's hypothesis which posits that species capable of uniparental reproduction through self-compatible breeding systems, and especially through spontaneous self-pollination, are more likely to be the dominating colonizers in new ranges following widespread dispersal (Baker, 1967, Pannell and Barrett, 1998).

Other studies using similar and/or identical methods employed for assessing these three study *Canna* taxa also support Baker's predictions. For example, Hao et al. (2011) in China has shown that ten of the twelve invasive species of Asteraceae they studied are fully autogamous, being able to set seeds though autonomous self-fertilization. Moreover, Rambuda and Johnson (2004) also found that all of the invasive alien plants they have tested were self-compatible, with some fully autogamous or capable of reproducing asexually through apomixis. Furthermore, it has been showed that South African Iridaceae naturalized elsewhere have a higher frequency of autonomous reproduction than species that have been introduced elsewhere without becoming invasive (van Kleunen and Johnson, 2007b). Although the abovementioned studies show support for Baker's hypothesis, other studies show that other colonizing species can be self-incompatible (e.g., Carr et al., 1986, Parker, 1997).

It has been estimated that there are more than 1000 hybrids within the genus *Canna* (Prince, 2010, Gupta et al., 2013), but to my knowledge, this study is the only one that has empirically assessed the potential of hybridization within this genus using controlled pollinations. The results of this study suggest that all study taxa are inter-fertile, however, this ability varies with which taxon is the sire of seeds of hybrids (Fig. 4 B, 5 B, 6 B). Plants intermediate between *C. indica*, *C. glauca* OF and YF were observed and anecdotally identified as hybrids (M. Cheek and M.S. Sibiya observations). Introduced *Canna* species in southern Africa often flower simultaneously. Therefore, hybridization of the study species is less likely to be limited by divergence in flowering time which is known to contribute to reproductive isolation in sympatric species (Ellis and Johnson, 1999, Ugoletti et al., 2013). Indeed, Ugoletti et al. (2013) showed that overlap in flowering time of the invasive *Impatiens glandulifera* and the less invasive *Impatiens balfourii* in Britain and Ireland resulted in successful pollination of these species by *Apis mellifera*, *Bombus hortorum*, and *B. pascuorum* which all switched between these species when foraging for pollen. Moreover, as all these study taxa have an overlap of floral visitors (M. S. Sibiya personal observations)*,* of which honey-

bees are definite pollinators, this overlap could be important in facilitating hybridization, as reported for milkweeds (Apocynaceae, Asclepiadoideae, *Gomphocarpus* species) in Australia (Ward et al., 2012) and invasive *Impatiens* species in Britain and Ireland (Ugoletti et al., 2013). The results of this study indicate that there are few genetic barriers to hybridization among the taxa, at least in terms of seed production. Some of the interspecific crossing combinations were, however, less successful (Fig. 4 B and 6 B). This was also the case with crosses among *Satyrium* orchid- (Ellis and Johnson, 1999) and *Impatiens* species in South Africa and in Britain and Ireland respectively (Ugoletti et al., 2013). Nevertheless, species of *Canna* have a long history of hybridization in horticulture (Maas-van de Kamer and Maas, 2008), and even our study taxa may not represent pure species, but have some hybrid background. Hybridization can complicate efforts at management as it introduces new forms and hybrids may not be biologically controlled by agents specific to parent species (see failure of control of *Salsola tragus* genotypes by introduced moths in Schierenbeck and Ellstrand, 2009). In addition, hybridization itself may alter breeding systems. For example, in my study system, hybridization with *C. indica* may transfer genes for autogamy into the otherwise allogamous species *C. glauca*.

Although selfing can increase seed production by alleviating pollen limitation, the resulting progeny may not be as fit as crossed progeny if there is inbreeding depression (Barrett and Harder, 1996, Barrett, 2002, Lin et al., 2012), and this may ultimately limit the spread of invasive species. Rapid seedling emergence has been proposed to likely increase species establishment and abundance (van Kleunen and Johnson, 2007b) and ultimately naturalization and invasiveness. Thus, it is important to germinate selfed seeds and compare the performance of seedlings to those from crossed seeds (Ward et al., 2012). In this study, I have found that *C. indica* seeds from selfing do not germinate as readily as seeds from crossing and selfed seedlings also performed relatively poorly. This result was unexpected since this species is autogamous and would be expected to have purged its genetic load. However, studies suggest that *C. indica* in its home range is allogamous and inbreeding depression could be explained if there has been a recent shift to selfing. In contrast, inbreeding depression was less evident in *C. glauca* OF, despite being allogamous and thus probably having a mixed mating system. Absence of inbreeding depression means that selfed seed can contribute to propagule pressure.

Vegetative reproduction may be important for plant species in new ranges, particularly if they are not capable of selfing or have not co-opted local pollinators (van Kleunen and Johnson, 2007a). The results from this study indicate that both study species are capable of vegetative reproduction, as is well known for *Canna* species (Maas-van de Kamer and Maas, 2008). In China it has been found

that self-incompatible Asteraceae which are invasive were capable of vegetative reproduction (Hao et al., 2011). Vegetative reproduction means that populations can be formed from discarded garden plants and not just from seeds. The importance of seeds versus vegetative reproduction for establishment of *Canna* populations is not yet known.

Conclusions

The results of this study are consistent with Baker's hypothesis about the capacity for uniparental reproduction in colonizing species. I have found that *C. indica* is partially autogamous and self-compatible. However, selfed progeny showed indications of inbreeding depression. I have also found that both colour forms of *C. glauca* are self-compatible to some degree and selfed progeny perform almost as well as crossed progeny. Hybridization among these species and forms means that new hybrid combinations will arise continuously in the invasive range and this is likely to lead to further evolution of the breeding systems. It would be interesting to test other *Canna* cultivars that are in horticulture but not invasive whether they are less capable of uniparental reproduction than are the study taxa.

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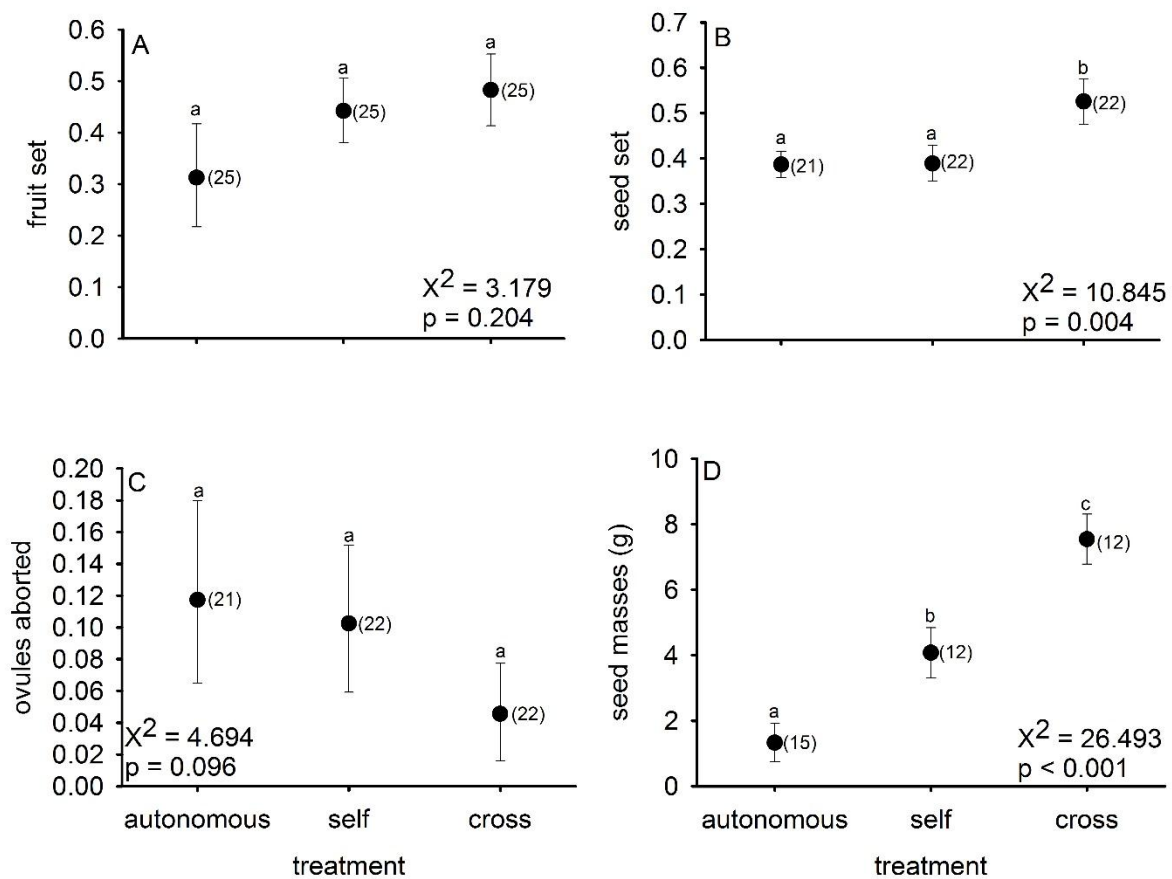
Figure 1: Mean values (± SE) of breeding system treatments of *C. indica* during 2014/15. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit for only 2015. Values in brackets represent total number of whole (with all flowers) plants treated. Means that share letters are not significantly different.

Figure 2: Mean values (± SE) of breeding system treatments of *C. glauca* OF during 2014/15. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit for only 2015. Values in brackets represent total number of whole (with all flowers) plants treated. Means that share letters are not significantly different.
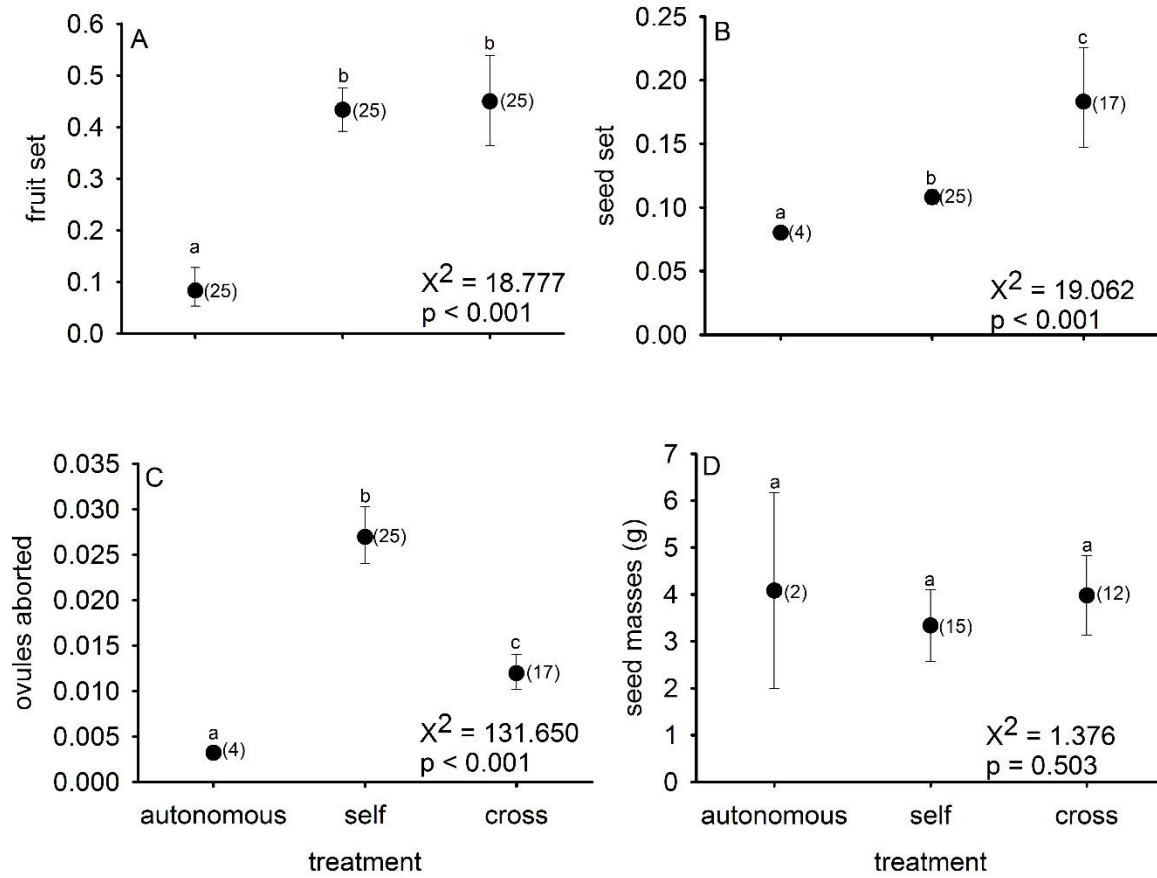
Chapter 3

Honeybees are the primary pollinators of New World *Canna* species naturalized in southern Africa

Abstract

Most flowering plants depend on animal vectors to effect fertilization. Introduced angiosperms, especially those with specialized pollination syndromes, are likely to experience limitations to fruit and seed set in new ranges owing to the rare occurrence and/or absence of pollinators. Pollinators and factors limiting fecundity were examined in *Canna indica* and two colour forms of *C. glauca* in their introduced range in South Africa. These *Canna* species are native to the Americas and adapted for pollination by hummingbirds. We observed visits of sunbirds that robbed nectar from the floral tubes and honeybees that collect pollen and also feed on nectar from holes left in the corolla by sunbirds. Experimental exclusion of birds did not affect seed production. Pollen-collecting honeybees appear to be the primary pollinators of the study taxa and deposited pollen on the stigmas in single visit experiments. Single visits by honeybees were also shown to enhance seed production in *C. indica*. Fecundity was pollen-limited in *C. indica* in one of two years of study and also in *C. glauca* YF. These results show that honeybees can act as substitute pollinators of exotic plant species adapted for pollination by hummingbirds.

Key words: Apis mellifera, *invasive alien species,* Canna, *pollinator limitation, pollination syndromes, hummingbirds*

Introduction

Over 308 000 species of angiosperm rely on animal vectors for movement of pollen between or within individuals for sexual reproduction and seed set (Ollerton et al., 2011, Dáttilo and Rico-Gray, 2018).The dependency of angiosperms on animal vectors can either be facultative or obligatory (Mayer et al., 2011). Some angiosperms are visited by a variety of animal vectors, while others

restrict visitation to only a specific taxonomic group of animals (Richardson et al., 2000, Traveset and Richardson, 2006); e.g., pollination of species of *Dalechampia* and *Clusia* by bees only in the families Megachilidae and Apidae (Pan et al., 1998). Pollination by a single species is relatively rare, good examples being *Ficus* (e.g., many figs are only pollinated by a single wasp species), *Yucca* (pollinated by *Tegiticula* moths), and some orchid species (the deception of wasps by orchid species) (Richardson et al., 2000, Johnson, 2005, Stout et al., 2006, Armbruster, 2012). Flowering plants introduced to new ranges are usually considered advantageous as they often leave behind natural enemies that inhibit their fitness in native ranges (Larson et al., 2002); however, they also often leave behind their pollinators and this can pose a barrier to seed production in the introduced range especially if they are specialized plants (Parker, 1997, Stout et al., 2006).

The reproductive success of introduced angiosperms, especially allogamous species, can depend on how well they integrate into prevailing pollination webs and on how well they attract local efficient pollinators (Geerts and Pauw, 2009, Harmon-Threatt et al., 2009). Introduced plants, particularly those with specialized pollination syndromes in their native ranges are likely to experience pollinator limitation and may only reproduce successfully if they can shift to new pollinators (Rodger et al., 2010, Coombs and Peter, 2010). Examples of this phenomenon in South Africa include *Nicotiana glauca* (Geerts and Pauw, 2009), *Acacia saligna* (Fabaceae) (Gibson et al., 2012, Gibson et al., 2013), and *Araujia sericifera* (Apocynaceae-Asclepiadoidea) (Coombs and Peter, 2010). Most invasive plant species have generalized pollination systems and are thus fairly likely to find substitute pollinators in their introduced ranges (Stout et al., 2006, Traveset and Richardson, 2006, Coombs and Peter, 2010).

Fecundity of introduced plant species is frequently pollen-limited, even when substitute pollinators are available (Richardson et al., 2000, Bufford and Daehler, 2014). This can be due to a shortage of pollinators or a shortage of mating partners in the case of self-incompatible species. For example, the obligatory outcrossing *Cytisus scoparius* (Fabaceae) in the USA experienced pollen-limitation in one of two sites due to shortage of pollinators (Parker, 1997). In the west of Ireland, where *Rhododendron ponticum* (Ericaceae) is declared invasive, some populations of this species suffered limited fruit and seed set owing to infrequent floral visitors (Stout et al., 2006). In one meta-analysis, it has been found that introduced plants incapable of autogamous reproduction suffered more pollen limitation than did native species (Burns et al., 2011). Reproductive success of introduced plant species thus usually depend on pollination mutualisms established in new ranges (Parker, 1997, Richardson et al., 2000, Stout et al., 2006).

Previous studies have shown that the success of invasive alien plant species in new ranges can be due to the establishment of mutualisms with honeybees, even in places where honeybees are not native (Hanley and Goulson, 2003, van Kleunen et al., 2007a, Zenni et al., 2009, Coombs and Peter, 2010, Gibson et al., 2012, Gibson et al., 2013). For example, *Centaurea solstitialis* L., which is invasive in the USA experienced significantly lower levels of seed set when honeybees were excluded from flower heads (Hanley and Goulson, 2003). In South Africa, *Acacia paradoxa* relies mainly on *Apis mellifera* susbspp. *capensis* for reproductive success and adequate seed production (Zenni et al., 2009). Flowers of *Datura stramonium* (Solanaceae) have been reported to be visited mainly by honeybees in South Africa (van Kleunen et al., 2007a). Honeybees are, indeed, highly generalist insects (Allsopp et al., 2008). Honeybees generally occur in significant numbers in plants they visit and this means that they can contribute to seed set even when they are not very effective at depositing pollen on a per-visit basis (Botes et al., 2009).

Species of *Canna* L. (Cannaceae) have been frequently reported to be pollinated by hummingbirds in their native ranges (Maas-van de Kamer and Maas, 2008, Prince, 2010). For example, it has been reported that *Canna indica* L. native in Argentina is pollinated efficiently by *Heliomaster furcifer* (Glinos and Cocucci, 2011). *Canna paniculata* in its native range is pollinated by *Phaethornis eurynome* (Maruyama et al., 2015) and *Canna glauca* L. is visited and possibly pollinated by moths, butterflies, honeybees and hummingbirds (Armbruster et al., 1999, Aizen et al., 2016). Despite *Canna* species seemingly being pollinated mainly by hummingbirds, Rambuda and Johnson (2004) reported *C. indica* to set seeds prolifically in southern Africa where this species is declared invasive and where hummingbirds are absent. *Canna indica* in southern Africa has been reported to be autogamous (Rambuda and Johnson, 2004) and allogamous in its native range (Glinos and Cocucci, 2011); however, the pollination biology of this species has not been assessed in its introduced range. Therefore, the occurrence of *Canna* species in southern Africa provides an opportunity to assess the role of pollinator substitutions in mitigating or facilitating the spread of invasive alien plant species.

In this study, I assess and document the pollination biology of three *Canna* taxa that are naturalized in South Africa. The taxa studied are *Canna indica* L., and two colour forms of *Canna glauca* L. *Canna* species are native to the New World (Soares et al., 2005, Baran et al., 2010), but they now occur widely across the tropics (Tobar-Vargas et al., 2013), including southern Africa (Maas-van de Kamer and Maas, 2008), owing to their use as ornamentals in horticulture (Young, 1982). Most *Canna* species are presumed to be self-compatible in their native ranges (Maas-van de Kamer and Maas, 2008) and they are afforded adequate pollination by hummingbirds (Maas-van de Kamer and

Maas, 2008, Glinos and Cocucci, 2011, Maruyama et al., 2015). Preliminary observations of naturalized *Canna* populations in South Africa by the author suggested that sunbirds do not feed legitimately from flowers (they pierce the floral tube) and that the only insects to make contact with the stigma are pollen-collecting honeybees. Honeybees are native in southern Africa (Donaldson, 2002) and are extreme generalists in terms of flower selection (Hanley and Goulson, 2003, Allsopp et al., 2008). I therefore predicted that the study taxa would either rely on selfing or be effectively pollinated by honeybees. Given the high levels of fruit set recorded in preliminary observations, I also predicted that fecundity is not pollen-limited in these species.

2. Methods

2.1. Study species

Canna is the only genus within the family Cannaceae (Zingiberales) (Maas-van de Kamer and Maas, 2008, Almeida et al., 2013, Mishra et al., 2013). *Canna* encompasses approximately 10 - 20 species (Kress and Specht, 2006, Mishra et al., 2013) and more than 1000 hybrids (Prince, 2010, Gupta et al., 2013). *Canna indica* L. is native to South America and occurs up to Mexico (Glinos and Cocucci, 2011). *Canna indica* occurs in shady, often wet forests, particularly along rivers or road sides in secondary vegetation (Maas-van de Kamer and Maas, 2008). The species has flowers with petals that are fused at the base and petals are further fused with the stamen and 3 staminoids (Glinos and Cocucci, 2011). Flowers tend to be red or deep orange (Chapter 1: Fig. 1 C). The distinctive feature of this species is that the lower sides of leaves are free of hairs (Maas-van de Kamer and Maas, 2008). *Canna glauca* L. is native to the Caribbean in South American as far South as northern Argentina (Maas-van de Kamer and Maas, 2008). In this study, two forms of *C. glauca* are studied; one form with orange flowers, here termed *C. glauca* OF (Fig. 1 A, D and E), while the other form has yellow flowers and is termed *C. glauca* YF (Fig. 1 B and C). In overall, *C. glauca* is found in marshes, swamps, and along margins of lakes (Maas-van de Kamer and Maas, 2008). *Canna glauca* usually has leaves that are dull grey, green, or bluish in colour (Maas-van de Kamer and Maas, 2008). All species of *Canna* are characterized by secondary pollen presentation process (Howell et al., 1993). In this event before anthesis, pollen from the anther is deposited under the receptive part of the stigma (Glinos and Cocucci, 2011).
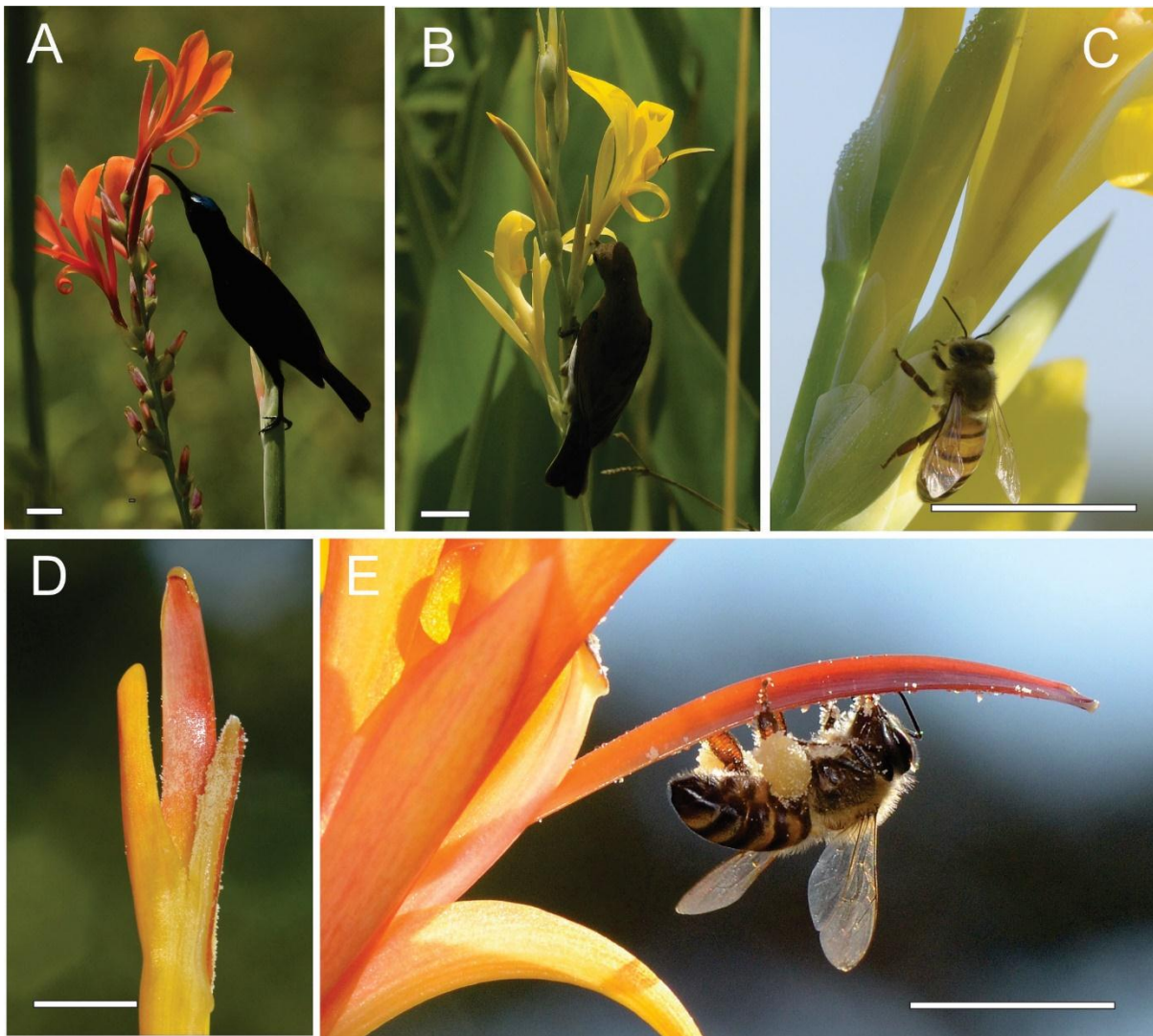
Figure 1: Flowers and animal visitors of the *Canna* study taxa. *C. glauca* OF flowers being robbed of nectar by an amethyst sunbird (A). *C. glauca* YF flowers being robbed of nectar by a white-bellied sunbird (B) and honeybee (C). *C. glauca* OF with pollen on anther (D) and with pollen being collected by a honeybee (E). Scale bar = 1 cm. All photos by S. D. Johnson.

2.2. Study Sites

Field observations and experiments were conducted in 2014 (February to October), 2015 (February to October), 2016 (December) and 2017 (March to June) in five sites around Pietermaritzburg, South Africa. The site in Hayfields (S 29° 38' 9.035" E 30° 25' 5.998") has populations of two forms of *C. glauca* that occur sympatrically with *C. indica* in a wetland in savanna vegetation. The sites in Gladys Manzi Road (S 29° 38' 29.998" E 30° 25' 10.001") and in intersection of St. Patricks and Leinster Road (S 29° 37' 4.998" E 30° 23' 34.998") has populations of *C. indica* alone in an open grassland near the road. *Canna glauca* OF and *Canna glauca* YF were also studied in Foxhillspruit

Canal near Shell fuel station (S 29° 21' 50.418" E 30° 13' 57.489") and in Msunduzi River in Boshoff Street (S 29° 36' 28.998" E 30° 23' 32.999") respectively, both in wet grassland vegetation next to a river.

2.3. Observations of floral visitors

Observations were conducted in 2015, 2016 and 2017 to assess floral visitors of each taxon. All observations were carried out specifically from 7h00 to 12h00 as 10-minute intervals. In all observations, the observer recorded the number of visitors, the identity, and behaviour of each floral visitor in a selected population.

Canna indica was observed every day for 20 days in Hayfields (December 2016), and for 20 days in Gladys Manzi Road (March and April 2017). *Canna glauca* OF was observed for 30 days (10 days for each of April, May 2015 and March 2017) and *C. glauca* YF for 10 days (in March 2017).

2.4. Single-visit effectiveness of bees

To assess the efficiency of honeybees in depositing *Canna* pollen on virgin stigmas, controlled single visit experiments (in 2015, 2016, and 2017) using flowers bagged prior to anthesis were conducted on all taxa. Single visit studies for *C. indica* were conducted in Hayfields (December 2016), Gladys Manzi Road, and St. Patrics-Leinster Road in March and May 2017 respectively. Single visit studies for *C. glauca* OF and *C. glauca* YF were carried out in all sites with these taxa in April to May 2015 and March 2017 respectively during flower handling.

Previously bagged flowers were exposed to single visits by honeybees. Stigmas were then dabbed on a block of Fuschin gel on a microscope slide to collect pollen, making sure that pollen from the pollen presenters did not fall on the block. The pollen-gel mixture on the slide was covered with a clean coverslip and the slide heated briefly so to melt the gel and dye the grains. Control virgin stigmas (unvisited) were also treated as described above as a control for autonomous self-pollination and pollen contamination from presenters.

To compare the efficiency of solitary bees in depositing *Canna* pollen on virgin stigmas, controlled experiments were conducted at the Gladys Manzi Road site in May 2017 using *C. indica* flowers bagged prior to anthesis. These experiments were done without controls, however. We used this species for this experiment as it was observed to be visited by both honeybees and solitary bees, but both visitors were not compared in terms of their efficiencies.

In all single visits to flowers, the number of pollen grains in slides from control and bee-visited flowers were counted systematically with a light microscope. Foreign pollen grains were counted but excluded in analyses.

To assess the contributions of honeybees to fruit and seed set, controlled single visit pollination experiments were conducted in all taxa in March and May 2017 using flowers bagged prior to anthesis. Virgin stigmas were allowed one-time contact with honeybees and then re-bagged and labelled accordingly. Virgin stigmas for controls were not visited, but were subjected to the same bagging procedure. The number of single visit and control stigmas per taxon was 10 and 10 respectively.

After four weeks, fruits were harvested and the proportion of flowers setting fruit was calculated. For each fruit, the number of viable and aborted seeds, as well as number of unfertilized ovules were counted on a dissecting microscope (A. Krüss Optronic – Germany; MSZ5000-T-IL-TL Stereo Microscope). Seeds from each fruit were weighed using an electronic balance.

2.5. Selective exclusion of sunbirds

To assess the contribution of birds to fruit and seed set, controlled selective exclusion experiments were performed by enclosing flower bud in mesh cages (mesh diameter ~ 20 mm). Wire cages allowed insect-visitors, but excluded birds.

These experiments were performed on *C. indica* and *C. glauca* OF in the Hayfields populations in 2014/2015. Whole plants (*C. indica*, 20 and 15 plants; *C. glauca* OF, 26 and 20 plants for each year respectively) were assigned to an open control or to a mesh-enclosed treatment. Following wilting of all flowers in individual plants, mesh cages were removed and fruits were allowed to develop. Fruits and seeds were counted and weighed as described previously.

2.6. Pollen supplementation experiments

To assess whether study taxa experience limitations to fruit and seed set due to pollen limitation, I used data from controlled hand-pollinations of bagged flowers (Chapter 2) to test whether hand-pollination increases seed production beyond the level achieved by natural pollination

In 2014 and 2015 (hereafter, 2014/15), only *C. indica* and *C. glauca* OF received all treatments in Hayfields. Whole plants (*C. indica*, 45 and 30 plants; *C. glauca* OF, 45 and 41 plants for each year

respectively) were assigned to the following treatments; (1) open natural pollination to test for natural levels of fruit and seed set; (2) supplementary self-pollination using pollen from the same test plants; and (3) supplementary cross-pollination using donors that were at least 10 m away from the test plants and usually in a different patch as a positive control.

In 2017, all taxa received the treatments. In this case, however, a split-plot design was used, where three flowers were used on each of 15 plants and each flower on a plant was assigned to a different pollen supplementation treatment.

2.7. Data analysis

All analyses were carried out using Generalized Linear Models (GLM) implemented in SPSS 24 (IBM Corp). In cases where data were potentially correlated, as in the case of flowers on the same plants, I used Generalized Estimating Equations (GEE). Subjects in GEEs included year in 2014/15 supplementation and bird exclusion treatments, plant no. in 2017 supplements and single visit pollination treatment, and plant no. in single visit pollen-deposition data. All proportions (e.g., flowers setting fruits, aborted ovules, ovules setting seeds) were analysed using a model, where the probability distribution was binomial and the link function was logit. The predictors were as follows; in 2014/15 and 2017 supplement treatments, the predictors were the supplementation treatments. In 2014/15 bird exclusion treatments, the predictors were controls and bird exclusion treatments. In single visit deposition treatments, the predictors were controls and bee visited treatments.

Canna pollen-deposition data were analysed using a negative binomial distribution with a log link function. Total number of seeds per fruit (hereafter seed mass) was analysed using a Gaussian distribution and identity Link function. Significance testing in all GLMs used likelihood ratios, except in the case of GEEs which used Score statistics or, in the case of small samples sizes, Wald statistics.

3. Results

3.1. Observations of floral visitors

During 2016 (December) and 2017 (March and April), a total of 108 floral visitors were observed foraging on *C. indica*. Twenty three visitors were honeybees (only observed in Hayfields) and 85

were solitary bees (82 observed in Gladys Manzi Road and three observed in Hayfields). Twenty honeybees (86.9 %) were collecting pollen from the secondary pollen presenter (and all making contact with the receptive stigma) and on average (± SE) they spent 68.4 ± 5.2 s per flower. Three honeybees were drinking nectar (outside the base of floral tubes) and spent on average 22.3 ± 0.3 s per flower. Of the solitary bees seen visiting *C. indica*, 37 were collecting pollen (six in Gladys Manzi Road making contact with the receptive stigma while three in Hayfields did not) and on average they spent 45.7 ± 4.9 s per flower, 10 were seen drinking nectar (inside the base of floral tubes) for an average of 14.2 ± 2.3 s per flower, 34 were hovering while inspecting flowers (for 30.4 ± 5.4 s), and four were in aggressive interactions for 6.8 ± 3.1 s with ants already in the flowers of *C. indica*.

In 2015 (April and May) and 2017 (March), a total of 629 floral visitors were observed foraging on *C. glauca* OF that occurred only in Hayfields. Twelve visitors were sunbirds which fed on nectar on the outside base of floral tubes (two observed and this lasted for 35.0 ± 0.0 s per flower). Two solitary bees were observed hovering around these plants, and they did so for an average of 15.0 ± 5.0 s. Four wasps were observed, of which two were hovering in front of flowers for 23.5 ± 6.5 s and two drinking nectar from puncture holes at the base of flowers for 29.0 ± 18.0 s per flower. The remaining 611 floral visitors observed in the *C. glauca* OF population were honeybees. One hundred and seventy six honeybees were seen collecting pollen (and all making contact with the receptive stigma) for an average of 13.8 ± 2.5 s per flower, 371 were foraging for nectar (136 honeybees drinking inside floral tubes for 33.2 ± 5.1 s, and 235 drinking from holes pierced in the floral tubes for 22.5 ± 3.5 s per flower), and 64 were seen hovering in front of flowers for 23.3 ± 3.7 s.

In *C. glauca* YF, 25 floral visitors were observed foraging during March of 2017. One sunbird was observed drinking nectar outside the base of floral tubes for 35.0 s. The remaining visits were by honeybees. Sixteen honeybees were collecting pollen (and all making stigma contact for an average of 40.7 ± 8.0 s per flower), one was drinking nectar outside the base of floral tubes for 27.0 s, and seven were hovering in front of flowers for 9.7 ± 2.8 s.

3.2. Single-visit effectiveness of bees

In *C. indica*, there was no significant difference between the mean number of *Canna* pollen grains deposited by honeybees versus the controls on respective stigmas (Fig. 2 A). However, in *C. glauca* OF, flowers visited by honeybees had 56-fold more pollen grains than did controls (Fig. 2 B).

Similar results where obtained in *C. glauca* YF, where honeybees deposited 20-fold more pollen relative to the controls (Fig. 2 C).
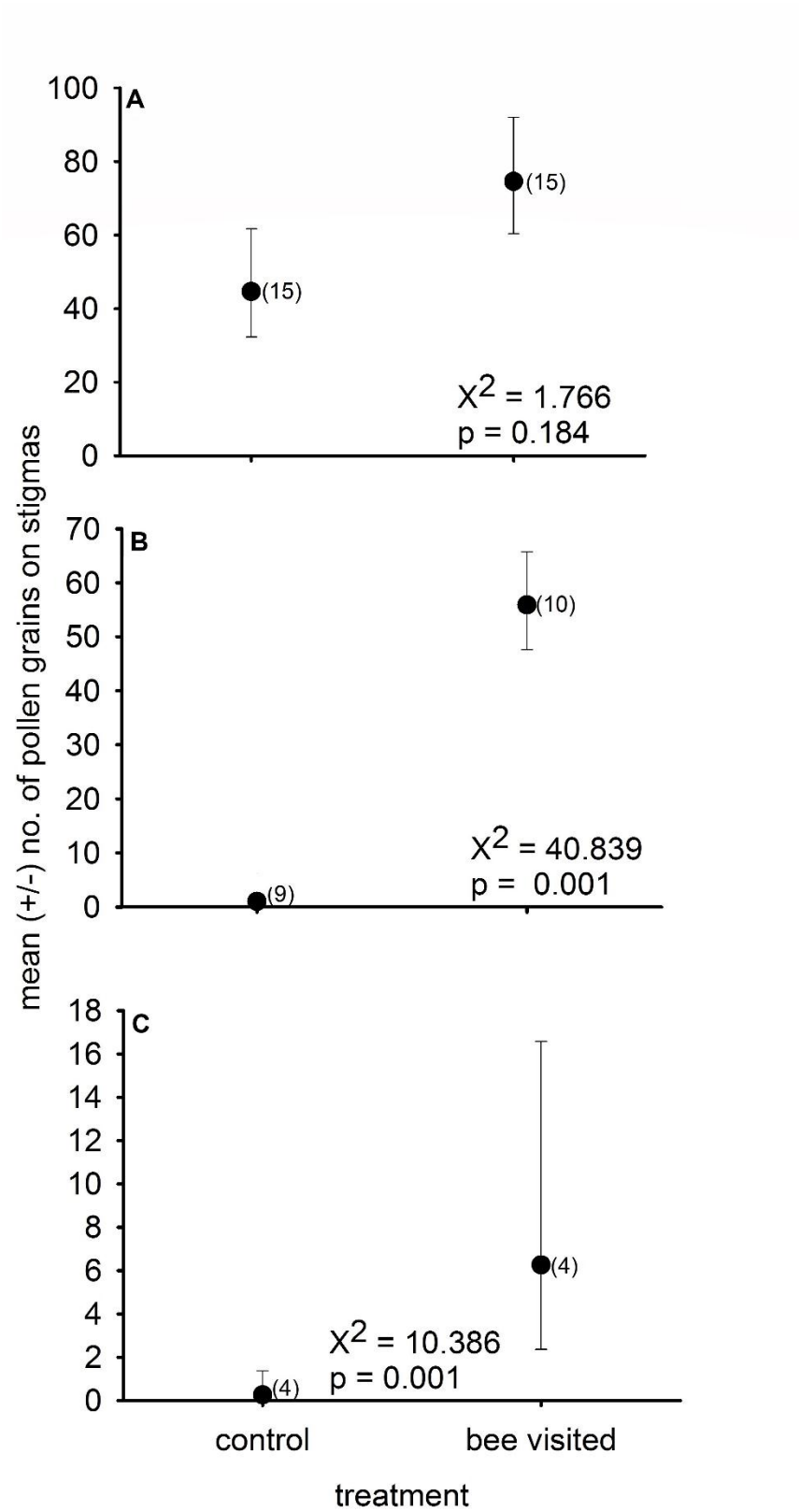

mean (+/-) no. of pollen grains on stigmas

A
(15)
(15)
$X^2 = 1.766$
$p = 0.184$

B
(10)
(9)
$X^2 = 40.839$
$p = 0.001$

C
(4)
(4)
$X^2 = 10.386$
$p = 0.001$

control   bee visited

treatment

Figure 2: Mean (± SE) number of *Canna* pollen grains deposited by honeybees on single visits in stigmas of *C. indica* (A), *C. glauca* OF (B), and *C. glauca* YF (C). Values in brackets represent sample sizes.

Only *C. indica* set fruits for both controls and honeybee single visit pollination treatments. Honeybee single visit pollination did not influence fruit set of *C. indica* (Fig. 3 A), but did result in slightly increased seed set (Fig. 3 B), reduced ovule abortion (Fig. 3 C) and a higher mass of seeds (Fig. 3 D).
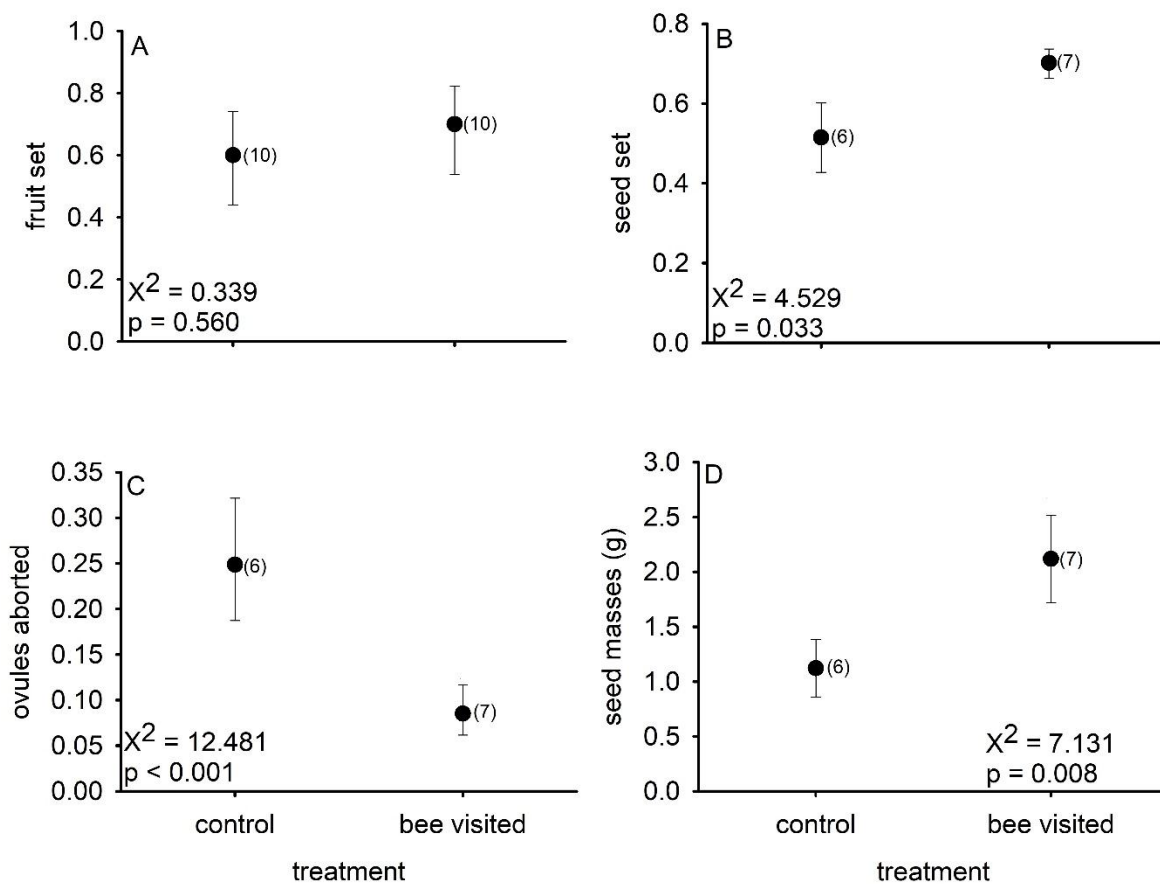

Figure 3: Mean values (± SE) of contributions of honeybees to fruit and seed set of *C. indica*. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated.

3.3. Selective exclusion of birds

The exclusion of birds from flowers of both *C. indica* (Fig. 4) and *C. glauca* OF (Fig. 5) resulted in slightly increased seed set (but did not affect fruit set) relative to when birds were allowed to visit flowers.
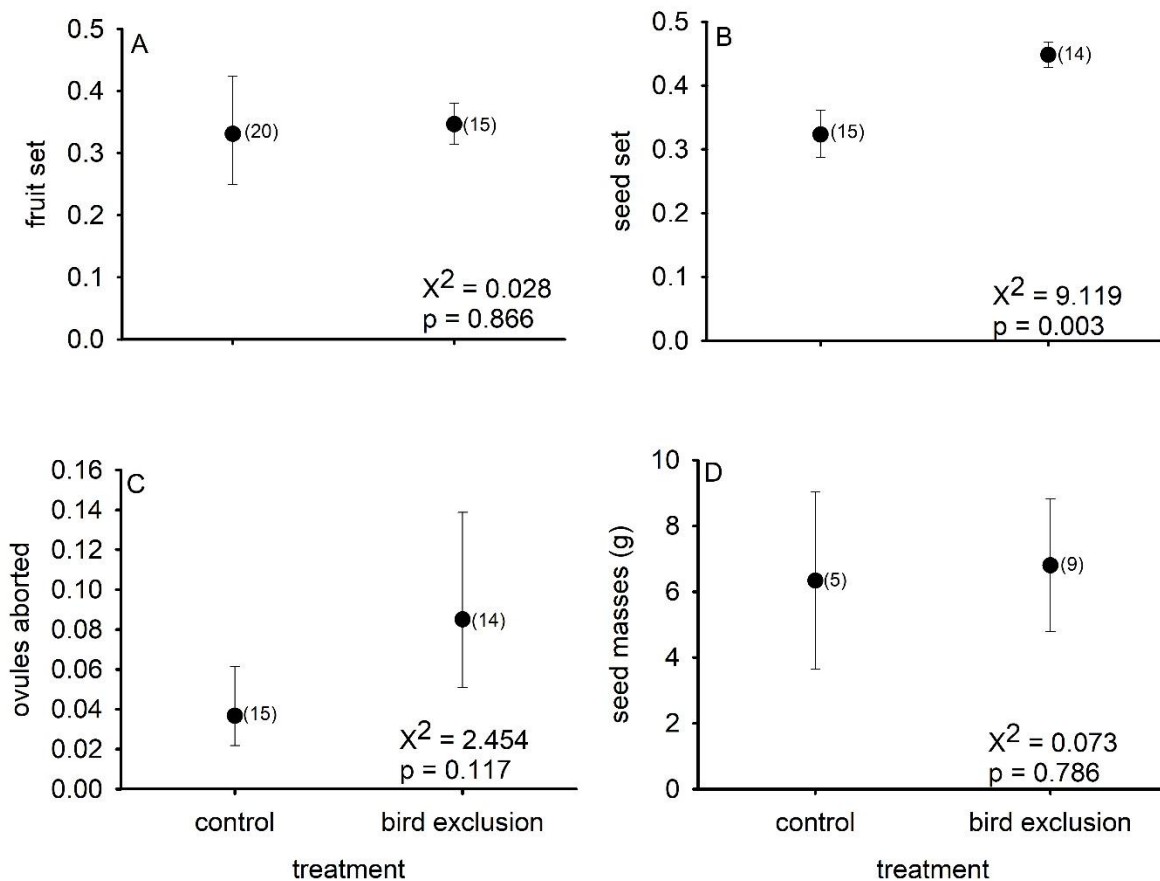

Figure 4: Mean values (± SE) of contribution of sunbirds to fruit and seed set of *C. indica* in 2014/15 whole plant treatments. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit for only 2015. Values in brackets represent total number of whole (with all flowers) plants treated.

Figure 5: Mean values (± SE) of contribution of sunbirds to fruit and seed set of *C. glauca* OF in 2014/15 whole plant treatments. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit for only 2015. Values in brackets represent total number of whole (with all flowers) plants treated.
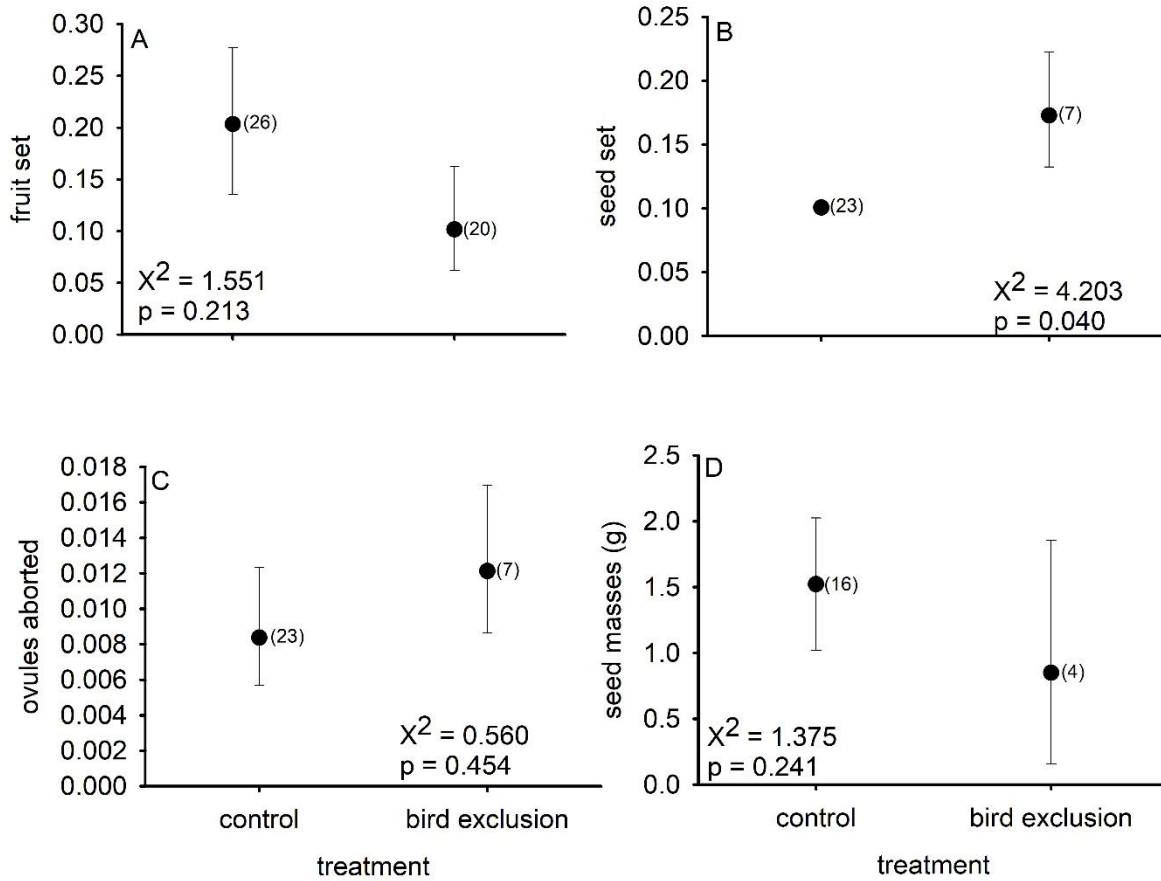
3.4. Pollen supplementation experiments

In 2014/15, hand-pollination with cross and self-pollen increased fruit and seed set of *C. indica* (Fig. 6 A and B). In addition, abortion rates from open-pollinations and supplementary cross-pollinations were lower (Fig. 6 C).

In 2014/15, the hand cross-pollination treatments, in relation to open-pollinations, had no significant effect on fecundity of *C. glauca* OF (Fig. 7 A, to D).
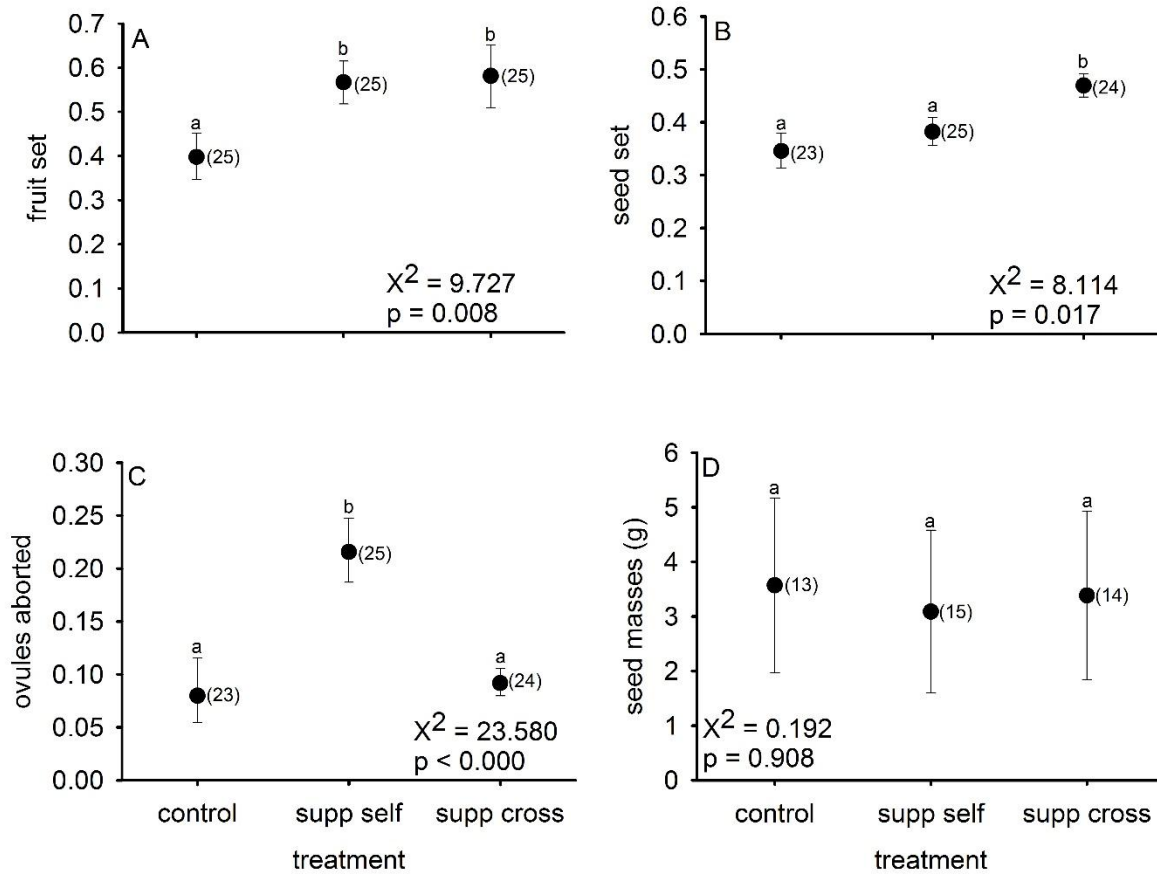
Figure 6: Mean values (± SE) of fruit and seed set of *C. indica* in situations of limited floral visitors in 2014/15 whole plant treatments. Supp self = supplementary self-pollination, Supp cross = supplementary cross-pollination. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit for only 2015. Values in brackets represent total number of whole (with all flowers) plants treated. Means that share letters are not significantly different.

Figure 7: Mean values (± SE) of fruit and seed set of *C. glauca* OF in situations of limited floral visitors in 2014/15 whole plant treatments. Supp self = supplementary self-pollination, Supp cross = supplementary cross-pollination. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit for only 2015. Values in brackets represent total number of whole (with all flowers) plants treated. Means that share letters are not significantly different.
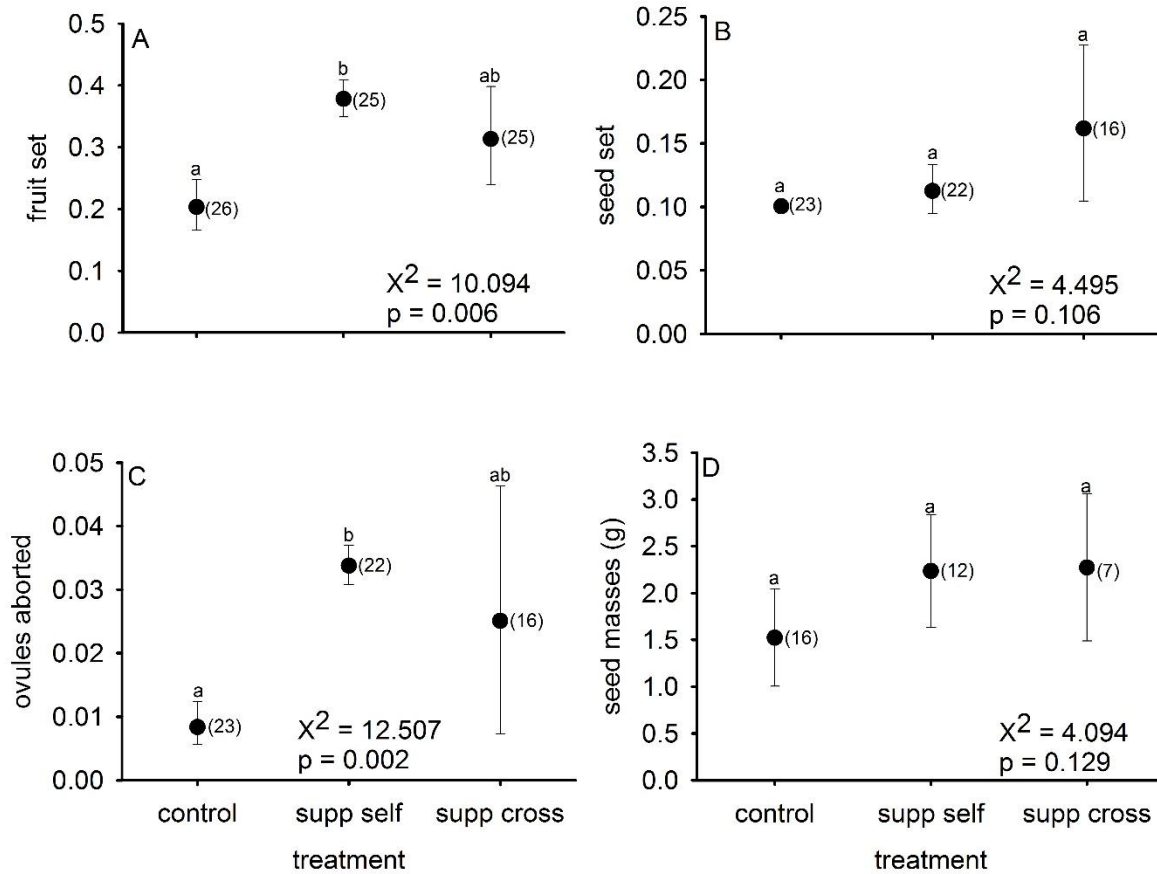
Relative to open-pollination, hand-pollination with cross and self-pollen did not increase fecundity of *C. indica* (Fig. 8 A - D) or *C. glauca* OF (Fig. 7 A - D), but did increase fecundity of *C. indica* (Fig. 8 B and D) in 2017.

Figure 8: Mean values (± SE) of fruit and seed set of *C. indica* in situations of limited floral visitors in 2017 split plot design. Supp self = supplementary self-pollination, Supp cross = supplementary cross-pollination. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated. Means that share letters are not significantly different.
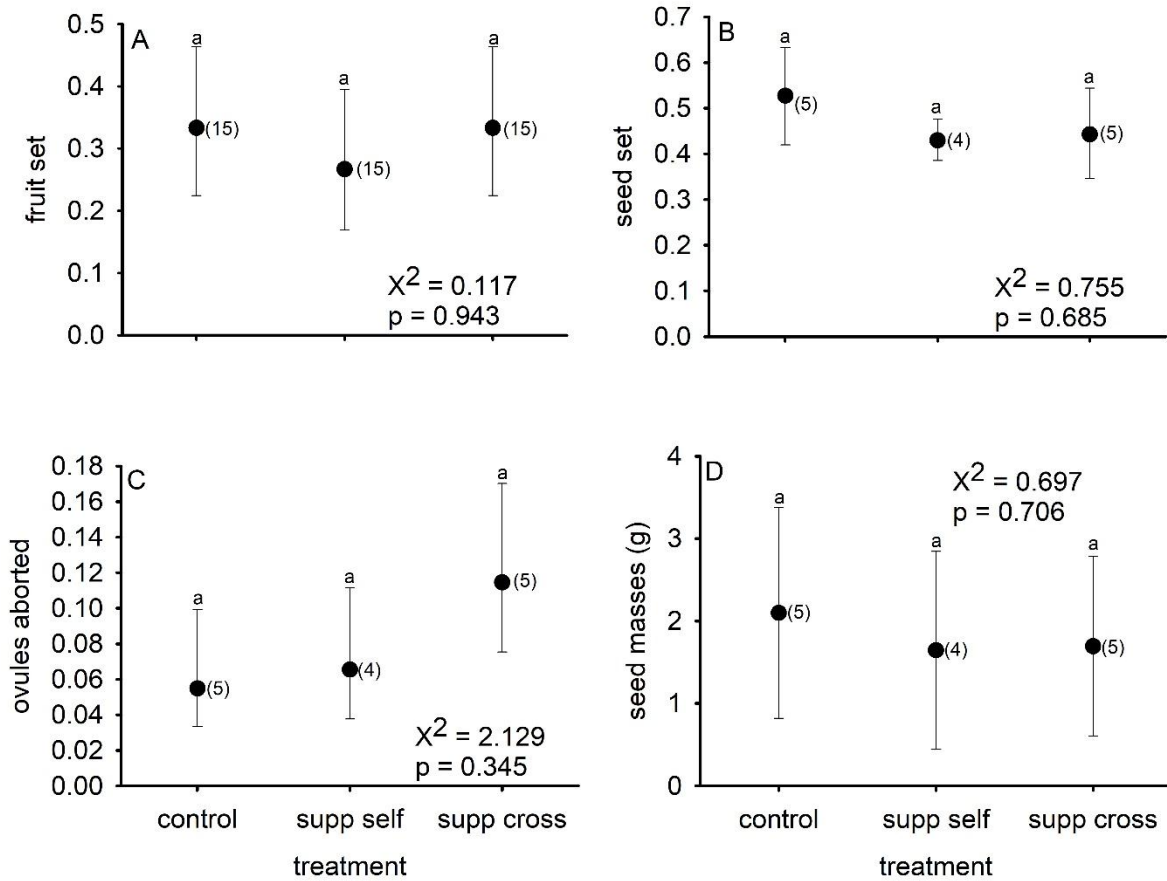
Figure 9: Mean values (± SE) of fruit and seed set of *C. glauca* OF in situations of limited floral visitors in 2017 split plot design. Supp self = supplementary self-pollination, Supp cross = supplementary cross-pollination. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated. Means that share letters are not significantly different.

Figure 10: Mean values (± SE) of fruit and seed set of *C. glauca* OF in situations of limited floral visitors in 2017 split plot design. Supp self = supplementary self-pollination, Supp cross = supplementary cross-pollination. Fruit set (A) is the proportion of flowers setting fruits. Seed set (B) represents proportion of ovules setting seeds per fruit, ovules aborted (C) represents proportion of aborted ovules per fruit, and seed masses (D) represents total mass of seeds per fruit. Values in brackets represent number of plants (with one flower) treated. Means that share letters are not significantly different.
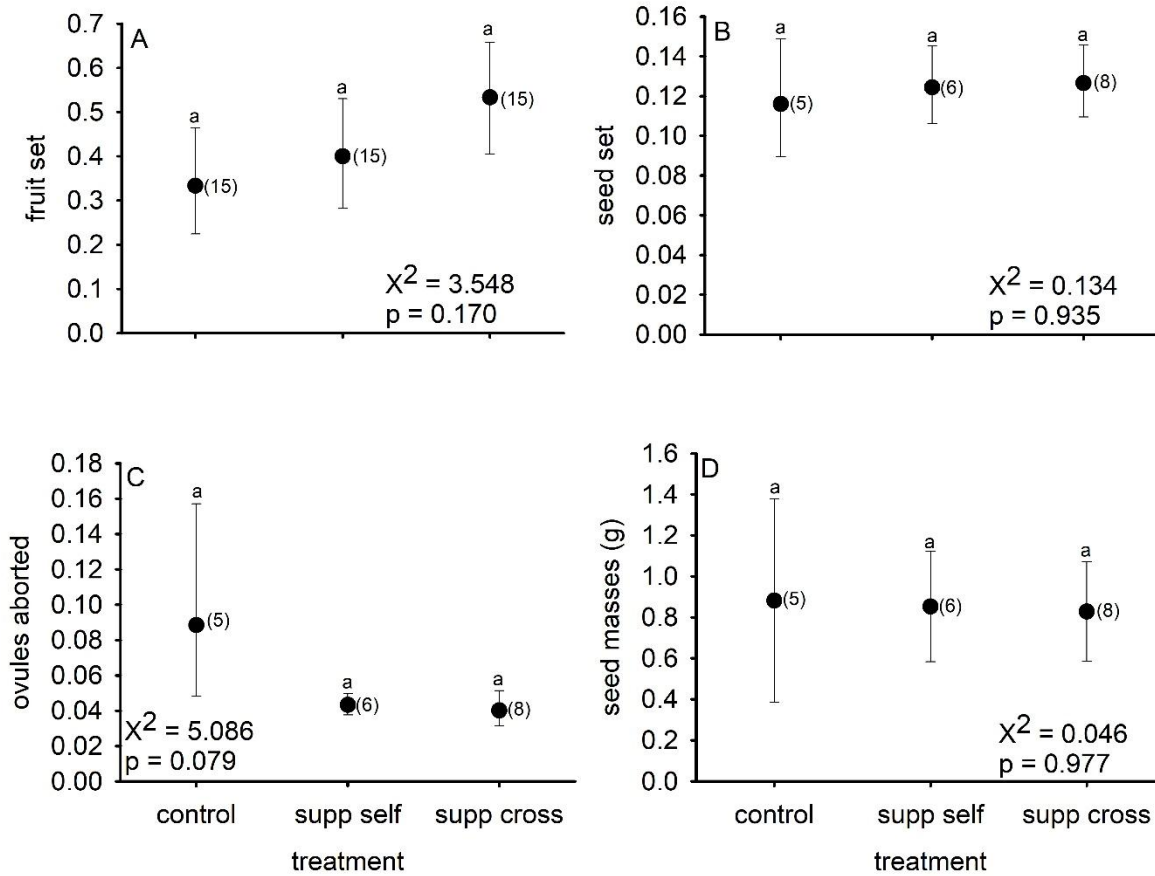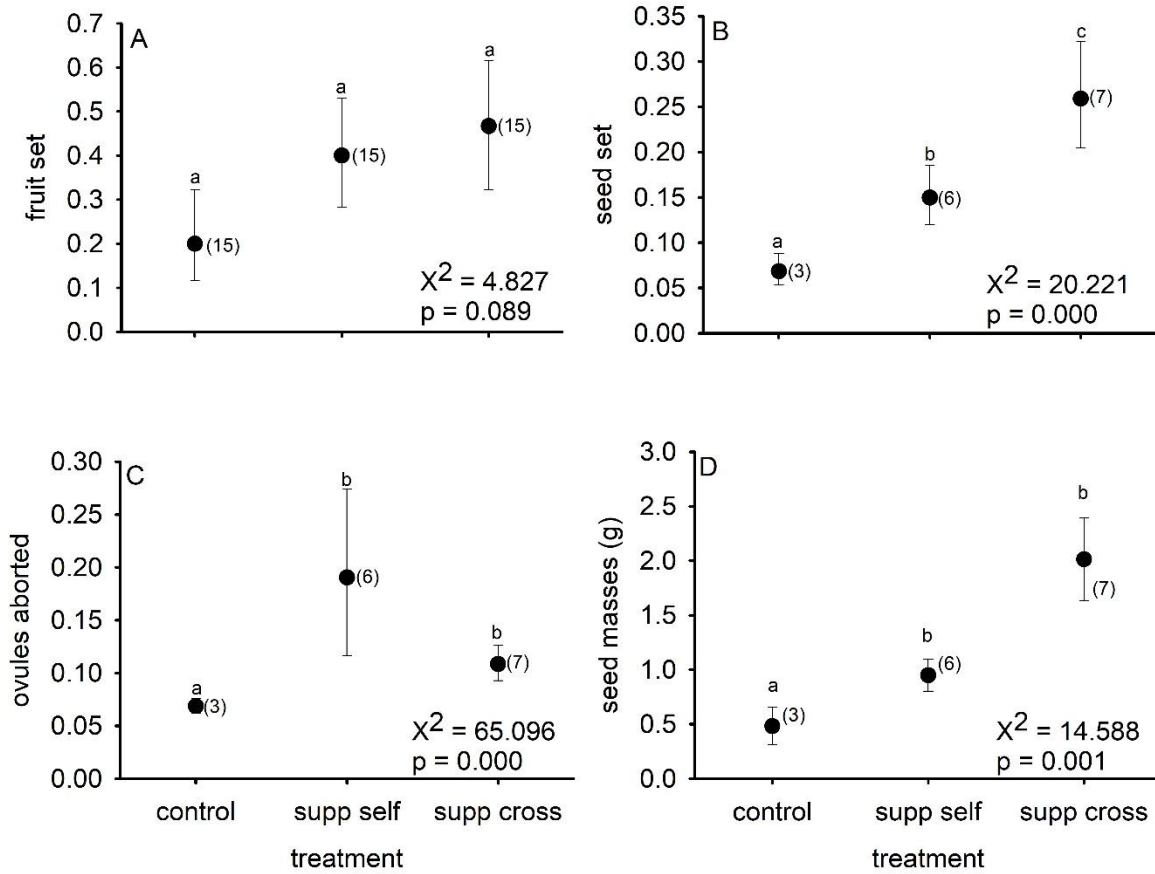
Discussion

In this study, I have found that naturalized *Canna* species are visited by sunbirds, honeybees and solitary bees. However, sunbirds rob flowers of nectar by piercing the corolla. The results of this study suggest that honeybees are effective pollinators of *C. glauca* as they clearly contributed to pollen deposition on stigmas of *C. glauca* (Fig. 2 B and C). Of the honeybees foraging for pollen on *C. indica*, 20 bees made clear stigma contact. However, we did not detect additional deposition of

Canna pollen besides that arising from automatic self-pollination in this species (Fig. 2 A). However, visitation by honeybees did slightly increase fecundity of *C. indica* (Fig. 3 B, C and D).

Literature documenting the role of honeybees in plant reproduction often mentions that they are inefficient as pollinators (Richardson et al., 2000, Westerkamp and Gottsberger, 2000, Traveset and Richardson, 2006), particularly in bird-pollinated systems (Botes et al., 2009). However, many studies have implicated honeybees as legitimate pollinators of invasive alien plant species in introduced ranges (Richardson et al., 2000, Hanley and Goulson, 2003, Traveset and Richardson, 2006), particularly in southern Africa (Coombs and Peter, 2010, Gibson et al., 2011, Gibson et al., 2012, Gibson et al., 2013). Furthermore, it has also been mentioned that their widespread occurance in many habitats may increase chances that obligatory outcrossing and/or specialized introduced plant species may be afforded at least some pollination (Richardson et al., 2000). In this study, honeybees afford adequate pollination to the study taxa. In Grahamstown, the native honeybee *A. mellifera* was shown to pollinate the invasive "moth catcher" *Araujia sericifera*. This South American invader has white, dense, scented flowers throughout the day and night which were shown to be mainly visited and pollinated by honeybees during the day and moths only making negligible contributions to fitness of this plant species (Coombs and Peter, 2010). This was also the case with *A. saligna* in southern Africa. *Acacia saligna* has significantly co-opted native honeybees (*A. mellifera* subspecies *capensis*) away from the native species *Roepera fulva* (Gibson et al., 2012), and these honeybees are anecdotally considered to effect cross-pollination (Gibson et al., 2012, Gibson et al., 2013). Other invasive acacias benefiting from the prevalence of honeybees in southern Africa include *A. paradoxa* which has been observed in the Table Mountain Park being pollinated by *A. mellifera* subspecies *capensis* (Zenni et al., 2009). In addition, other species that may benefit from the widespread occurance of honeybees in South Africa include *Datura stramonium* of which has *A. mellifera* as its primary visitor and is an effective pollinator (van Kleunen et al., 2007a).

In this study, sunbirds could have been expected to be co-pollinators with honeybees as the study taxa are adapted for pollination by hummingbirds (Maas-van de Kamer and Maas, 2008, Glinos and Cocucci, 2011). Sunbirds in the Old World efficiently pollinate *Nicotiana glauca*, another invasive species adapted for pollination by hummingbirds in the New World (Geerts and Pauw, 2009). However, my observations and experimental results suggest otherwise. In this study, the exclusion of sunbirds, but not honeybees, from flowers resulted in *C. indica* and *C. glauca* OF setting significantly more seeds (Fig. 4 B and 5 B), perhaps because birds damage flowers when robbing the corolla. This is a strong suggestion that sunbirds make no significant contribution to fruit and

seed set of these species. Although my results are contradicting that of Geerts and Pauw (2009), e.g., honeybees contributing more significantly than sunbirds to seed set of *C. indica*, sunbirds in this study system rarely visited *C. indica* (M.S. Sibiya personal observation). In addition, sunbird's rare visits to *C. indica* (in 2014/15) was only destructive – sunbirds poked the outside base of floral tubes when foraging for nectar and often resulting in freshly opened flowers (with pollen and stigmas still intact) detaching from the ovary.

Sunbirds also did not make a significant contribution to fruit and seed set of *C. glauca* OF. This may be in part because of their infrequent visits, robbing behaviour or due to their destructive nature. Although these taxa have red to orange colouration that conforms to the bird pollination syndrome (Ford et al., 1979, Johnson and Nicolson, 2008, Botes et al., 2009, Geerts and Pauw, 2009), I did not measure nectar content and concentration within these plants as this is one other characteristic that is associated with ornithophilous pollination syndromes (Johnson and Nicolson, 2008). Thus, further reseach may investigate whether sunbirds' attraction to these plant taxa is associated with availability of resources.

It is often mentioned that seed production in angiosperms relying on animals for reproduction is generally limited by floral visitors which are inefficient pollen vectors, e.g. deposit insufficient number of pollen grains of low quality (Brys and Jacquemyn, 2011). Limitation to seed set in introduced plant species in new ranges is one barrier that limits their spread, and understanding this may improve our knowledge of invasions and development of improved management strategies (Bufford and Daehler, 2014). By examining the effects of pollen limitation due to infrequent or inefficient floral visitors on fecundity, in this study I have found that the autogamous *C. indica* during the years 2014/15 experienced pollen limitation to fruit and seed set (Fig. 6 A and B). In addition to pollen limitation, I have found that low quality supplies of additional pollen by floral visitors compromises the fecundity of this species (Fig. 6 C). However, in 2017 the autogamous *C. indica* experienced no limitation to fruit and seed set (Fig. 8 A and B) caused by infrequent or inefficient floral visitors, and quality of pollen delivered had no effect on fecundity (Fig. 8 C and D). *Canna glauca* OF in 2014/15, and in 2017 experienced no limitation of fruit and seed set (Fig. 7 A and B; Fig. 9 A and B), but low quality pollen slightly compromises the overall reproductive output of this taxon (Fig. 7 C). *Canna glauca* YF in 2017 had seed set, but not fruit set, limited by availability of pollen (Fig. 10 A and B). Furthermore, offspring quality of this taxon is highly affected by the quality of siring pollen (Fig. 10 D).

Due to the fact that *C. indica* is autogamous, it was never expected that this species would suffer from pollen limitation in any year because autogamy may be an additional source of pollen (Rambuda and Johnson, 2004, van Kleunen et al., 2007a, van Kleunen et al., 2007c, Hao et al., 2011). Comparison of hand-pollinated and open-pollinated flowers suggest that autogamy is only partially effective in this species. Notably, the *C. indica* populations were relatively small and were comprised of seven – 100 individuals whereas other taxa had populations with 250 (*C. glauca* YF) and 500 (*C. glauca* OF) individuals each (M. S. Sibiya personal observations). Plants of *C. indica* received comparatively low honeybee visits (23 in two months) in relation to other taxa which had 176 (*C. glauca* OF in three months) and 24 (*C. glauca* YF in one month) of visits. Indeed, small populations are more likely to experience pollen limitation due to relatively low number of pollinators that visit them (e.g., Allee effects Stout et al., 2006, van Kleunen et al., 2007a). *Canna indica* may have suffered pollen limitation in 2014/15 due to infrequent honeybee visits in small populations regardless of the capacity of autogamous reproduction which may be essential for initial colonization (Rambuda and Johnson, 2004). *Canna glauca* OF may have not suffered from pollen limitation in all years because its populations are continuous with adequate number of available mates and received adequate pollinator visits. *Canna glauca* YF might have experienced limitation to seed set due to infrequent floral visitors because this taxon also had only one small patch which honeybees can visit. Indeed, limitation of seed set in plants due to insufficient pollinator visits is a common phenomenon (Parker, 1997). It has been documented that in 258 species, 160 had suffered reduced seed set due to pollinator limitation (Burd, 1994) and some populations of *R. ponticum* in Ireland where it is invasive experienced a reduction in fruit and seed set when pollinators were rare (Stout et al., 2006). In 2017, *C. indica* may have not experienced pollen limitation because of increased number of honeybees probably attracted to simultaneously flowering plants (M. S. Sibiya personal observations). The above phenomena is similar to that of Parker (1997) who found that *C. scoparius* in USA experienced pollen limitation in wild settings and not in urban sites because the broom in urban sites had significantly more pollinator visits that were attributed by other simultaneously flowering native species (Richardson et al., 2000). In addition, the differences in pollen limitation of *C. indica* during 2014/15 and 2017 may simply reflect that pollinator services vary in space and time, and that pollen limitation essentially decreases as pollinator availability increases (Parker, 1997) or simply that other factors such as resource availability may be essential for fruit establishment (Stout et al., 2006). Indeed, other factors, such as nectar availability, are associated with reproduction in flowering plants as has been shown that *Delphinium nelsonii* Greene (Ranunculace) increased it seed set when nectar was readily available in some individuals and seed set diminished to some individauls when nectar was not available (Zimmerman, 1983).

Conclusions

This study has documented the pollination biology and pollinator species of three *Canna* taxa that are invasive and naturalized in South Africa. This study has also inspired further research within these taxa. For example, sunbirds in South Africa efficiently pollinate *N. glauca* which also has hummingbird pollination syndrome (Geerts and Pauw, 2009), but do not seem to feed legitimately on *Canna* flowers and thus pollinate them, perhaps because of differences in hovering ability among sunbird species. In addition, further research can investigate the extent to which solitary bees contribute to fecundity of *C. indica* through measures of pollen deposition among solitary bees and controls and measures of fruit and seed production between autogamy and solitary bee single pollination. However, this study supports the idea that honeybees are important pollinators of invasive species in new ranges (Richardson et al., 2000, Coombs and Peter, 2010, Gibson et al., 2013). In this study, honeybees were found to deposit significant numbers of pollen grains on stigmas to efficiently pollinate two forms of *C. glauca*. In addition, honeybees may be important in pollinating *C. indica* as they increased some aspects of fecundity of this species. In general a combination of autonomous self-pollination (in *C. indica*) and effective pollination by honeybees (in *C. glauca*) meant that very little pollen-limitation of fecundity was evident in the study taxa.

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CHAPTER 4

General discussion and conclusions

Invasive alien plant species are a major threat to biodiversity (Kumschick et al., 2017a), economy (Kumschick et al., 2017b), human health (Bandara et al., 2010), and scarce water sources of infested areas (Abella et al., 2009), and if left unmanaged, overall negative impacts may be exacerbated (Wilson et al., 2013). Owing to these crippling impacts, there is a need to identify species characteristics that are correlated with invasiveness (Hao et al., 2011), and these can prove invaluable for mitigating impacts and/or predicting future invasions (Rejmanek and Richardson, 1996). *Canna indica* L. (Cannaceae) is declared invasive in southern Africa (DoEA, 2009) but only one study has empirically assessed breeding systems characteristics of this species that are associated with invasiveness (Rambuda and Johnson, 2004). Reliable estimates of invasion risks of species require many other species traits associated with invasiveness; e.g., hybridization potential, extent of reliance on (specialized) pollinators for reproduction, and existence of invasive related taxa (Ugoletti et al., 2013, Nxumalo et al., 2016, Moodley et al., 2017). These traits are particularly important to assess because risk assessment protocols often lack this information (Panetta, 2016). In this thesis, I have assessed and documented the reproductive and pollination biology of three *Canna* taxa that are invasive and naturalized in South Africa. I have achieved this by studying the breeding systems, extent of inbreeding depression following selfing, hybridization potential, pollinators and their single-visit efficiencies, and the magnitude of pollen limitation. The aim of this chapter is therefore to summarise and discuss major findings about the role reproductive and pollination biology plays in facilitating the spread of our study taxa. This chapter also aims to aid in estimating risk of invasiveness and possibility of inclusion of these study taxa in management programs.

The role of breeding systems in facilitating spread of three studied *Canna* taxa

Along the lines of Baker's hypothesis that was coined in 1955 (Baker, 1967), the study taxa were hypothesized to exhibit self-compatibility and possibly also automatic self-pollination, owing to the occurrence of pollen closely with the receptive part of stigma (Glinos and Cocucci, 2011). I demonstrated that *C. indica* is indeed mostly autogamous and self-compatible (Chapter 2: Appendix, Fig. 1 A and B; Fig. 1 A and B). Further evidence for capacity of automatic reproduction

is highlighted in Chapter 3 where I demonstrated that automatic self-pollination delivers pollen to stigmas (Fig. 2 A). Rambuda and Johnson (2004) also demonstrated that *C. indica* in southern Africa is fully autogamous and self-compatible. However, inbreeding depression was detected in this species and this raises questions about the contribution of selfed seed to demographics of this species. Evidence for inbreeding depression is highlighted in Chapter 2 where I have demonstrated that selfed *C. indica* seeds germinate less readily than crossed seeds and have less vigour (Fig. 7 A – D). Another line of evidence for inbreeding depression in this species was that visits by honeybees which presumably contributed outcross pollen to stigmas resulted in production of higher quality seeds of heavier masses and reductions in ovule abortions in comparison to those seeds arising from automatic self-pollination (Chapter 3: Fig. 3 D and C).

Canna glauca YF is self-compatible but incapable of automatic self-pollination (Chapter 2: Fig. 3 A and B). Further support for this is highlighted in Chapter 3 where I have shown that negligible amounts of pollen are deposited in stigmas through automatic self-pollination whereas considerable amounts of pollen are deposited by honeybees (Fig. 2 C). Inbreeding depression was not detected, but I cannot exclude it being important at later stages of plant development.

Canna glauca OF is also incapable of automatic self-pollination, and is partly self-compatible (Chapter 2: Fig. 2 A and B; Appendix, Fig. 1 A and D). Supporting evidence is also highlighted in Chapter 3 where I have shown that honeybees outperform deposition in control treatments where automatic self-pollination is the only mechanism of pollen deposition to stigmas (Fig. 2 B). While this taxon sets fruits and seeds with self-pollen, I have shown that outcross-pollen often results in greater seed production and seeds of higher quality (Chapter 2: Fig. 2 B and D; Appendix, Fig. 1 B and D). Despite being partially self-compatible, this taxon produces progeny with self-pollen that does not experience inbreeding depression (Fig. 7 A – E).

In this thesis, self-compatible breeding systems are prevalent in the study taxa and are likely to contribute to the spread of these taxa across the invasive range South Africa. *Canna indica* has a potential to deploy considerable amounts of seeds without the aid of pollinators though automatic self-pollination, but the fate of selfed progeny in contributing to spread is less likely owing to extensive levels of inbreeding depression expressed as poor performing progeny. Contrary to this, both *C. glauca* taxa are more likely to spread despite their incapability to self-pollinate automatically, as they have co-opted local native honeybees as pollinators

The role of pollinators in facilitating spread of three studied *Canna* taxa

In this thesis, I have also hypothesized that our study taxa are afforded efficient pollination by honeybees and thus do not suffer from pollen limitation to fruit and seed set. I demonstrated that honeybees (together with solitary bees) are the most frequent visitors to all study taxa. In addition, honeybees are the only animal pollinators that often effect pollination of all study taxa by making positive stigma contact (Chapter 3, section 3.1). Evidence for effectiveness of honeybees as cross-pollinators of *C. indica* is highlighted in their contribution to fruit and seed set where I have shown that their pollination results in significantly high seed production, of high quality (Chapter 3: Fig. 3 B and D). I demonstrated that sunbirds make no contribution to fruit and seed set in either *Canna* species and act only as nectar thieves. Indeed, their presence seems to reduce seed production, perhaps on account of damage to flowers by their bills as they probe around the ovaries (Chapter 3: Fig. 4). As the primary pollinators of *Canna* species, native honeybees contribute to their seed production and thus invasiveness. Honeybees probably also facilitate hybridization among the study taxa which are largely inter-fertile (Chapter 2: Fig. 4, 5, and 6).

Although honeybees deposit pollen grains on stigmas (Chapter 3: Fig. 2), I found that a single honeybee visit to both forms of *C. glauca* did not result in fruit set (Chapter 3: section 3.2). This could reflect resource limitation at the time of that experiment or that multiple honeybee visits to stigmas are required for effective pollination.

Future research directions and concluding notes

Overall, these results are consistent with Baker's hypothesis that self-compatible breeding systems are favoured and prevalent in colonizing species (Rambuda and Johnson, 2004). Selfing may be important for initial establishment of populations and initial spread from sites of introductions to occupy available habitats (Pannell et al., 2015a, Pannell, 2015). Follow up studies are required to elucidate the actual level of selfing in these species. This could be achieved using co-dominant molecular markers such as SSRs (Henry, 2012). Estimate of outcrossing rates using molecular markers would also allow independent marker-based estimates of total inbreeding depression. Another useful application of SSRs would be to estimate the extent of clonality in populations due to vegetative spread (Zane et al., 2002).

These results add to the growing evidence that many invasive alien plant species in new ranges are efficiently pollinated by honeybees (Coombs and Peter, 2010, Gibson et al., 2012). Indeed, all our study taxa demonstrate a potential for spread by seeds away from sites of introductions owing to the widespread occurrence of native generalist honeybees in South Africa (Donaldson, 2002, Allsopp et al., 2008).

It is clear that the study taxa pose considerable risk to vulnerable wetland habitat. A combination of ability to self and effective pollination by ubiquitous honeybees means that there are few barriers to seed production in these taxa and that new more invasive hybrid combinations are likely to arise in the future. Management is cost-effective when a potentially invasive species is detected early and objective control measures are implemented early enough to prevent a species from expanding its range and impacts (Le Roux et al., 2010). In this thesis I have provided data to aid in estimating risk of invasion (as per Australian Weed Risk Assessment Protocol Nxumalo et al., 2016) and attributes of these species to aid in decision making.

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