

國立臺灣大學生態學與演化生物學研究所

博士論文

Institute of Ecology and Evolutionary Biology

College of Life Science

National Taiwan University

Doctoral Dissertation



比較咸豐草三個變種的生物特徵

以了解大花咸豐草在臺灣的入侵優勢

Comparisons of three varieties of *Bidens pilosa* to identify
what traits make the variety *radiata* invasive in Taiwan

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中華民國 103 年 7 月

July, 2014

致謝



本論文的完成，承蒙許多人的協助。對於幫助過我的人，我滿懷感激並盡量謹記在心。

首先，感謝指導教授高文媛老師。老師一直以來全方位地教導與提攜，讓學生無比地感謝與感動。在實驗材料與研究設備方面，老師無私且大方地全力支持，讓我可以完全不用擔心實驗材料、研究設備與經費來源。在實驗操作與論文撰寫方面，老師除了教我專業學科內容，並一次又一次地提醒我，不要忘記科學研究的論證邏輯，更不厭其煩地修改我那難以閱讀的英語文句。當我獲得榮耀時，老師更加開心；當我有缺失時，老師小聲提醒。此外，老師對研究永保熱情、對教學滿懷抱負、對行政認真負責、對學生用心關懷，更是我敬佩與學習的目標。除了對我滿懷包容與照顧，老師的愛也擴及我的家人，讓我能一個充滿愛與關懷的環境中，完成這本論文、完成我的學業。

感謝謝長富老師、陳淑華老師、林讚標老師、王震哲老師、郭耀綸老師，在繁忙的學期末，仍認真、詳細地審閱論文，並在論文的論述、用字等各方面，提供許多寶貴的建議，使我獲益良多，也讓這本論文的內容更加完善。看到老師們註記的初稿，滿懷的感謝湧上我心頭。此外，陳淑華老師與謝長富老師在博士班期間的關懷與提攜，我都謹記在心，並由衷感激。

實驗期間，侯金日老師熱情地帶著我們找尋實驗材料、陳建德老師提供試驗地、生命科學院科技共同空間陳香君老師、莊以君學姊在螢光顯微鏡的操作諮詢、楊亞臻小姐在掃描式電子顯微鏡的操作協助、李鳳鳴老師提供染色體染色藥品、黃玲瓏老師、胡哲明老師、王俊能老師借用照相顯微鏡與螢光顯微鏡、張菽娟小姐對溫室的管理，在此一併致謝。

在漫長的博士班研究期間，感謝實驗室伙伴們的協助與鼓勵：蔡孟穎協助配置製備玻片所需的藥品、黃涵靈協助種子採集、吳泰中在儀器操作的諮詢，還有佳娟、承穎、承泰、顯淳、怡清的陪伴與鼓勵，漢晨、麗智、譯汎、閔義在我博士班生涯的最後一年帶給實驗室青春活力。感謝生態演化所洪麗分學姊、曾好馨、官淑蕙在研究或生活上的交流與鼓勵、陳保元協助材料採集、楊月鈴助教與莊桂蓮技正在行政事務上的協助。

感謝我的家人，奶奶、爸爸、媽媽、弟弟一直以來的支持與關懷，是他們成就了我的人生。感謝公公李輝達先生、婆婆黃素慧女士提供試驗地，以及對我和小孩生活上的悉心呵護，讓我能無後顧之憂。感謝外子李承恩先生全方位的協助，包含製作論文中的地圖以及分擔各項生活雜務等，讓我在研究與生活的人生旅程上，都有人一路溫暖相伴。

最後，謹以此論文獻給我摯愛的雙親：黃正綱先生與王珍珠女士。

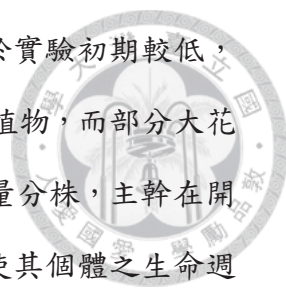
摘要



外來物種的入侵是造成生物多樣性降低的重要原因之一。根據臺灣植物誌的記載，臺灣有三種咸豐草變種：小白花鬼針、白花鬼針與大花咸豐草。這三者之中，大花咸豐草最晚在臺灣開始有採集紀錄。然而，自從大花咸豐草被引入臺灣後，在短短三十年內，已成為臺灣中、低海拔路邊、廢耕地或荒地中最常見的野草。為了了解究竟是什麼樣的生物特徵，使大花咸豐草被引入後，在臺灣的低海拔地區，比小白花鬼針與白花鬼針優勢，並成為臺灣嚴重的入侵植物，本研究比較此三種咸豐草變種的生長、繁育系統與生活史特徵。

在生物量的累積上，大花咸豐草、小白花鬼針與白花鬼針三者 in 夏季的相對生長速率均較秋季高，顯示高溫高光的夏季較適合此三種咸豐草變種生長。在夏季時，大花咸豐草的生長速率顯著高於另外兩變種；但在秋季時，三變種有相似的生長速率。由此推測：大花咸豐草相較於另外兩變種的生長優勢僅發生於高光高溫的環境中。此外，與另外兩變種相比，大花咸豐草具有較高的根部生物量比例、側枝的生長角度較為水平（較接近地面）、與較好的不定根生長能力，並具有二回側枝，這些特徵顯示大花咸豐草的營養繁殖潛力勝過另外兩變種。

研究三種咸豐草變種的開花生物學，發現大花咸豐草、小白花鬼針與白花鬼針三者均有次級花粉呈現與不完全雄先熟的現象，但在溫室中栽植時，只有大花咸豐草不會結瘦果，因此進行套袋實驗以了解其繁育系統。實驗結果確認大花咸豐草無法自交結瘦果，其繁育系統為自交不親和；而小白花鬼針與白花鬼針能於套袋中自交結瘦果，為自交親和。大花咸豐草有許多特徵於個體間有顯著差異，顯示大花咸豐草的高度異質性，可能是完全異交所造成的結果；而此特性也有助於大花咸豐草在不同的環境中生存、生長。



相較於另外兩變種，大花咸豐草雖然較晚開花，瘦果產量於實驗初期較低，但因小白花鬼針和白花鬼針植株在開完花後即死亡，為一年生植物，而部分大花咸豐草個體存活期間較長，並可透過無性繁殖的方式，產生大量分株，主幹在開花結果後，雖然會乾枯，但其分株可繼續生長、開花並結果，使其個體之生命週期可延長超過一年以上，並在栽植一年後，累積的瘦果產量超過另外兩變種。雖然小白花鬼針與白花鬼針所結瘦果較大花咸豐草瘦果重、發芽快，但三者最終發芽率並無顯著差異；且因大花咸豐草的瘦果較輕、較多，會比其它兩變種更有機會被動物攜帶、散播到遠方。

綜合實驗結果，我推論：大花咸豐草經由有性繁殖可以產生大量瘦果，這些瘦果因為有芒，容易吸附在動物身上，讓大花咸豐草可以擴散至四處。因為具完全異交的繁育系統，使得大花咸豐草具有高度異質性，能適應不同的環境，在許多不同棲地生存下來。而當大花咸豐草拓殖到一個新的棲地後，能透過無性繁殖，產生大量分株，使其在該地區能快速且持續地增加覆蓋面積。大花咸豐草兼具無性繁殖和完全異交的有性繁殖系統特徵，加上臺灣溫暖高光的氣候環境、土地利用的變遷，使得大花咸豐草能優於小白花鬼針與白花鬼針，成為臺灣嚴重的入侵植物之一。

關鍵詞：繁育系統、生長、大花咸豐草、入侵植物、生活史特徵

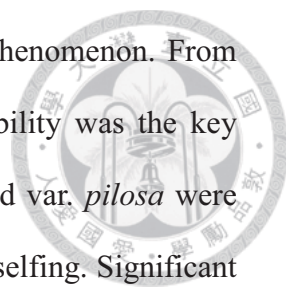
Abstract



The invasion of exotic species has become one of the most important factors reducing biodiversity. Three varieties of *Bidens pilosa*: var. *minor*, var. *pilosa* and var. *radiata*, were listed in the Flora of Taiwan. Among the three varieties, the first record of var. *radiata* in Taiwan was later than those of the other two varieties. However, after being introduced into Taiwan, var. *radiata* has become a common weed on roadsides, uncultivated field and waste grounds from low to middle elevations in the past three decades. In order to identify what traits let var. *radiata* dominant over the other two varieties and become one of serious invasive plants in Taiwan lowland now, I compared the growth, breeding system and life history traits of these three varieties.

The relative growth rates of the three varieties were higher in summer than in fall. The result suggested that summer with high light and temperature was more suitable for the growth of all three varieties than fall. In summer, var. *radiata* grew faster and accumulated more biomass than the other two varieties. Thus, var. *radiata* had growth advantage over the other two varieties in regions with high light and high temperature conditions. In addition, var. *radiata* had higher root/biomass ratio, more horizontally oriented axillary shoots, and grew more adventitious roots than the other two varieties. Among the three varieties, only var. *radiata* had secondary axillary shoots. With these characters, var. *radiata* had a greater potential of vegetative reproduction than the other two varieties.

It was found that all three varieties had secondary pollen presentation and were incompletely protandrous. However, when these three varieties grew sympatrically in a greenhouse, var. *minor* and var. *pilosa* produced achenes while var. *radiata* had no



achene set. Bagging experiments were conducted to explain the phenomenon. From the results of bagging treatment, I confirmed that self-incompatibility was the key mechanism preventing selfing in var. *radiata*, while var. *minor* and var. *pilosa* were self-compatible and were capable of producing achenes in bags by selfing. Significant differences were found in many traits among individuals of var. *radiata*. The result indicated that high heterogeneities in many traits of var. *radiata* might result from its obligate xenogamous breeding system.

Although var. *radiata* flowered later and set less achenes than the other two varieties in the beginning, half individuals of var. *radiata* lived longer and formed ramets by vegetative reproduction. These ramets flowered and set achenes when matured. Consequently, var. *radiata* accumulated significantly more achenes than the other two varieties after being planted for one year. In addition, although achenes of var. *minor* and var. *pilosa* were heavier and germinated earlier than those of var. *radiata*, the final germination percentage was not different among the three varieties. Having more and lighter achenes, var. *radiata* would be dispersed by animals more easily than the other two varieties.

In conclusion, massive achenes produced by sexual reproduction potentially allow var. *radiata* dispersing into far-reaching habitats. High heterogeneities were found in many traits of var. *radiata*, which might allow var. *radiata* to have widely fundamental niche. After established in a new habitat, var. *radiata* could generate many ramets by vegetative reproduction, expand horizontally, eventually occupied the area and became dominant. In summary, the breeding system and life history traits of var. *radiata*, in combination with the warm and high light climate and land use change in Taiwan confer var. *radiata* advantage over var. *minor* and var. *pilosa* and become

one of serious invasive plants in Taiwan.



Keywords: Breeding system, Growth, Hairy beggar-ticks (*Bidens pilosa* L. var. *radiata* Sch. Bip.), Invasive plants, Life history traits



Contents

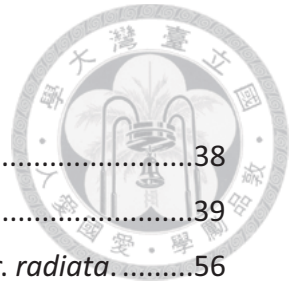


摘要	I
Abstract	III
Contents	VII
LIST OF FIGURES.....	IX
LIST OF TABLES.....	X
Chapter 1 General introduction	1
1-1 The definition of invasive species	2
1-2 What traits make plants invasive?	4
1-3 Studies of invasive plants in Taiwan.....	7
1-4 <i>Bidens pilosa</i> var. <i>radiata</i> and its congeners.....	10
1-5 Objectives and studies of the thesis	12
Chapter 2 A comparison of growth and morphology of the three varieties of <i>Bidens pilosa</i>, var. <i>minor</i>, var. <i>pilosa</i> and var. <i>radiata</i>, in two seasons	15
摘要.....	16
Abstract	17
Introduction	19
Materials and Methods.....	22
Results	24
Discussion.....	28
Figures and Tables.....	32
Chapter 3 Floral biology of <i>Bidens pilosa</i> var. <i>radiata</i>	41
摘要.....	42
Abstract	43
Introduction	44
Materials and Methods.....	46
Results	49
Discussion.....	52
Figures and Tables.....	56

Chapter 4 Breeding systems of the three varieties of <i>Bidens pilosa</i>, var. <i>minor</i>, var. <i>pilosa</i> and var. <i>radiata</i>	63
摘要.....	64
Abstract	65
Introduction	66
Materials and Methods.....	68
Results	72
Discussion.....	74
Figures and Tables.....	77
Chapter 5 Life history traits of the three varieties of <i>Bidens pilosa</i>, var. <i>minor</i>, var. <i>pilosa</i> and var. <i>radiata</i>	83
摘要.....	84
Abstract	85
Introduction	86
Materials and Methods.....	87
Results	89
Discussion.....	94
Figures and Tables.....	98
Chapter 6 Chromosomal number of populations of the three varieties of <i>Bidens pilosa</i>, var. <i>minor</i>, var. <i>pilosa</i> and var. <i>radiata</i>	105
摘要.....	106
Abstract	107
Introduction	108
Materials and Methods.....	110
Results and Discussion	111
Figures and Tables.....	114
Conclusions.....	117
Literature Cited	121

LIST OF FIGURES

Figure 2-1	The angle of axillary shoots of three varieties.....	38
Figure 2-2	Leaf area and SLA of three varieties..	39
Figure 3-1	Disc florets number in a capitulum of <i>Bidens pilosa</i> var. <i>radiata</i>	56
Figure 3-2	Disc floret and its reproductive components of <i>Bidens pilosa</i> var. <i>radiata</i>	57
Figure 3-3	The Six floral stages of <i>Bidens pilosa</i> var. <i>radiata</i> during anthesis.	59
Figure 3-4	Stigma receptivity of <i>Bidens pilosa</i> var. <i>radiata</i> at six floral stages.	60
Figure 3-5	Pollen viability of <i>Bidens pilosa</i> var. <i>radiata</i> at six floral stages.....	61
Figure 3-6	Summaries of six floral stages.	62
Figure 4-1	Fluorescent pictures of three types of pistils.	80
Figure 4-2	Pollen viability of three varieties at six floral stages.....	81
Figure 5-1	Capitulum and achene production of three varieties.	101
Figure 5-2	Accumulated capitulum and achene production of three varieties... ..	102
Figure 5-3	Survival rates of three varieties.	103
Figure 5-4	Germination rates of three varieties..	104
Figure 6-1	Sampling sites of three varieties.....	115
Figure 6-2	Somatic chromosomes of three varieties.	116



LIST OF TABLES

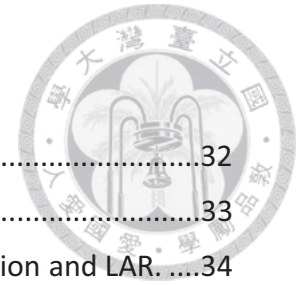
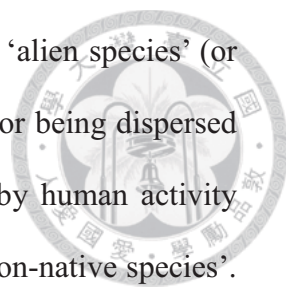


Table 2-1	Blooming of three varieties.....	32
Table 2-2	Growth, biomass allocation and LAR of three varieties.....	33
Table 2-3	Effect of season and variety on growth, biomass allocation and LAR.	34
Table 2-4	Shoot biomass allocation of three varieties.....	35
Table 2-5	Effect of season and variety on shoot biomass allocation.....	36
Table 2-6	Growth of adventitious root of three varieties.....	37
Table 4-1	Disc floret number and achene set of three varieties in bagging experiment.....	77
Table 4-2	Percentage of three pistil type of three varieties in bagging experiment.....	78
Table 4-3	Pollen/ovule ratio of three varieties.	79
Table 5-1	Blooming of three varieties.....	98
Table 5-2	Capitulum and achene production of three varieties.	99
Table 5-3	Achene/capitulum in three periods of three varieties.	100
Table 6-1	Disc floret number and achene set in hand pollination experiment....	114



Chapter 1

General introduction



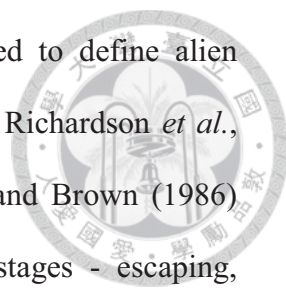
Species of a region can be classified into ‘native species’ and ‘alien species’ (or non-native species). Native plants are those plants evolved locally or being dispersed into the region by natural force. Plants introduced into a region by human activity either deliberately or unintentionally are called ‘alien species’ or ‘non-native species’. Among these alien species, about one-tenth of them could survive, generate offspring and establish new populations in the new geographic range and become ‘naturalized species’ (Williamson, 1993). Most naturalized species coexist virtually unnoticed with native species and cause no threat to the introduced areas. But about one-tenth of them largely increased their populations and spread fast, thus became dominant in the introduced regions (Williamson and Brown, 1986). Some researchers called these species ‘invasive species’.

Due to the globalization of human societies, more and more species have been transported by human activity and thus increase their distribution range and spread into new ecosystems. This situation lead the ratio of alien species versus native species gradually increase. Therefore, the overexpansion of these alien species have become one of the serious threatens to global biodiversity, ecosystem and economics nowadays (Begon *et al.*, 1996; Pimentel *et al.*, 2005). Taiwan has no exception.

In this chapter, I made a general introduction on the definition of invasive plants, what traits make plants invasive, and studies of invasive plants in Taiwan. Finally, a summary on the objective and studies of each chapter of this thesis were given.

1-1 The definition of invasive species

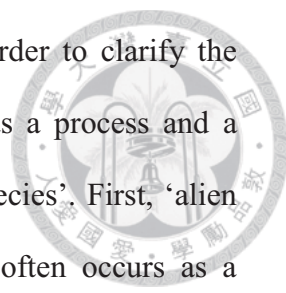
Invasion ecology has been proposed and studied for more than 50 years (Elton, 1958; Baker and Stebbins, 1965), terminology for alien species still has not reach



consensus and cause some confusion. Some researchers preferred to define alien species based on invasive process (Williamson and Brown, 1986; Richardson *et al.*, 2000b; Colautti and Maclasc, 2004). For example, Williamson and Brown (1986) suggested that the invasion success can be defined into three stages - escaping, establishing and becoming a pest. Alien species being at different stages of the invasion success were denoted as imported, introduced, established or naturalized and pest. ‘Imported’ cover alien species found in collections or accidentally brought into the country; ‘introduced’ are alien species found in the wild; ‘established’ or ‘naturalized’ include species already having a self-sustaining population; and ‘pest’ denote species having negative economic effect (Williamson and Brown, 1986).

Richardson *et al.* (2000b) used major barriers limiting the spread of alien plants to define the terms. The authors suggested that plants have been transported by humans across a major geographical barrier are ‘introduced’; plants that overcame geographic barrier, environmental barrier, and can reproduce regularly are ‘naturalized’. And if the naturalized plants could produce offsprings, often in very large numbers at considerable distance from parental plants (approximate scales: >100 m; <50 years for taxa spreading by seeds and other propagules) and thus have the potential to spread over a considerable area, are considered ‘invasive plants’ (Richardson *et al.*, 2000b). Colautti and Maclasc (2004) also proposed to use invasion stage to define non-indigenous species. For example, ‘invasive species’ means species has already been transported to the new region, and can survive, produce offspring, and disperse locally in the new region.

Invasive Species Advisory Committee (ISAC) suggested that an invasive species is a non-native species whose introduction does or is likely to cause economic or

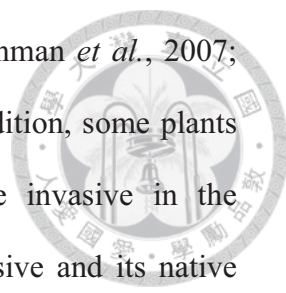


environmental harm or harm to humans, animals, or plants. In order to clarify the meaning, ISAC also suggested that invasion can be thought of as a process and a species must overcome several barriers to become an ‘invasive species’. First, ‘alien species’ were species overcoming a geographical barrier which often occurs as a mountain range, ocean, or similar physical barrier to movement of seeds and other reproductive plant parts. In the following, the alien species must overcome survival barriers, establishment barriers and dispersal and spread barriers, and then they were able to become ‘invasive species’.

In this study, I adopt ISAC’s definition and consider the widely distributed *B. pilosa* var. *radiata* as an invasive plant in Taiwan.

1-2 What traits make plants invasive?

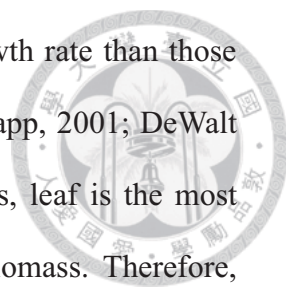
Not all alien plants were invasive plants. In fact, only very few proportion alien plants would become invasive. Successful invaders often have some traits promoting their invasiveness (van Kleunen *et al.*, 2010). What traits making plants invasive have interested many invasive ecologists (Price and Jain, 1981; Rejmánek and Richardson, 1996; Levine *et al.*, 2003; Sutherland, 2004; van Kleunen *et al.*, 2010). In order to find out these traits, researchers compared invasive and non-invasive plants (Rejmánek and Richardson, 1996; Pattison *et al.*, 1998; Sutherland, 2004; Zheng *et al.*, 2009; Ordonez *et al.*, 2010). Some traits were obtained by analyzing the data of invasive and non-invasive plants in database or in literature review (Rejmánek and Richardson, 1996; Sutherland, 2004; Hamilton *et al.*, 2005; Leishman *et al.*, 2007; Ordonez *et al.*, 2010; van Kleunen *et al.*, 2010). Some traits were clarified by comparing the invasive and non-invasive or native plants of a region (Pattison *et al.*,



1998; Baruch and Goldstein, 1999; Smith and Knapp, 2001; Leishman *et al.*, 2007; Ordonez *et al.*, 2010; He *et al.*, 2011; Scharfy *et al.*, 2011). In addition, some plants were not considered invasive in its native range, but become invasive in the introduced range. The difference between populations in its invasive and its native range were often considered as the key point. Therefore, the populations of plants in their native range and introduce range were often compared (DeWalt *et al.*, 2004). Furthermore, plants often shared many similar traits with their congeners, comparing the invasive plants with their non-invasive congeners might be easier to find out the important traits making plants invasive. Therefore, many research compared the traits of invasive plants and traits of their non-invasive congeners (McDowell, 2002; Burns and Winn, 2006; Grotkopp and Rejmanek, 2007; Zheng *et al.*, 2009).

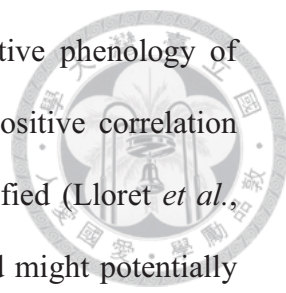
Though var. *radiata* was introduced into Taiwan later than var. *minor* and var. *pilosa*, within 30 years, var. *radiata* has become a serious invasive plant and dominant over the other two varieties in Taiwan lowland. Therefore, in this study, I compared the var. *radiata* and the other two varieties, var. *minor* and var. *pilosa*, to identify what traits making var. *radiata* more dominant than the other two varieties in Taiwan now.

Growth is one of the most relevant traits determining plant performance in the field. Plants growing rapidly would outcompete other sympatric species in resources uptake and become dominant. Many invasive plants were found growing faster and accumulating more biomass than non-invasive plants (Pattison *et al.*, 1998; DeWalt *et al.*, 2004; Grotkopp and Rejmanek, 2007; Zheng *et al.*, 2009; van Kleunen *et al.*, 2010). Biomass allocation plays an important role in regulating relative growth rate (RGR). It has been found that plants allocating more biomass to above ground



components (have higher shoot/root ratio) would had a faster growth rate than those with more allocating to roots (Pattison *et al.*, 1998; Smith and Knapp, 2001; DeWalt *et al.*, 2004; Zheng *et al.*, 2009). In all above ground components, leaf is the most important organ performing carbon assimilation to accumulate biomass. Therefore, leaf traits are considered important indicators in invasive plants (Baruch and Goldstein, 1999; McDowell, 2002; Leishman *et al.*, 2007; Zheng *et al.*, 2009; Ordonez *et al.*, 2010; Scharfy *et al.*, 2011). Specific leaf area (SLA) represents light-capturing area deployed per dry mass allocated. Therefore, SLA is closely correlated with other plant traits such as photosynthetic capacity, leaf N content, leaf life-span and relative growth rate. Most invasive plants were found having higher SLA than non-invasive plants (Baruch and Goldstein, 1999; Smith and Knapp, 2001; Hamilton *et al.*, 2005; Grotkopp and Rejmanek, 2007; Leishman *et al.*, 2007; Ordonez *et al.*, 2010; van Kleunen *et al.*, 2010; He *et al.*, 2011; Scharfy *et al.*, 2011). But still some invasive plants were found having lower SLA than non-invasive ones (McDowell, 2002; Zheng *et al.*, 2009).

Being able to reproduce outside the native range is essential for the establishment and spreading of introduced species (Richardson *et al.*, 2000a; Richardson, 2004; Sutherland, 2004; van Kleunen *et al.*, 2010). Accordingly, reproduction plays an important role in invasive process. Thus, to investigate the reproductive biology of invasive species is fundamental for understanding biological invasion. Many studies on the reproductive biology of invasive plants have recognized that breeding systems play a crucial role in contributing to the successful invasion of alien species into non-native ranges (Petanidou *et al.*, 2012; Ward *et al.*, 2012; Munguia-Rosas *et al.*, 2013). In general, self-compatible species were considered good colonizers because they could sexually produce offsprings without the presence of other neighbors

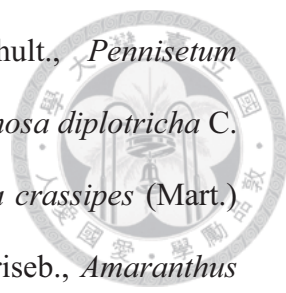


(Baker, 1955). In addition to the breeding system, the reproductive phenology of plants is also an important subject (Ghersa and Holt, 1995). A positive correlation between blooming time and local plant abundance has been identified (Lloret *et al.*, 2005). Having a longer flowering period and generating more seed might potentially confer the species ability to increase population size locally and to disperse widely and become invasive. Seed mass, seed size, and seed amount were also found different between invasive and non-invasive plants (Hamilton *et al.*, 2005; Ordonez *et al.*, 2010; van Kleunen *et al.*, 2010).

In addition, invasive plants were often found having vegetative reproduction ability which could increase local dominance and persistence quickly (Lloret *et al.*, 2005; Lasso *et al.*, 2009). Clonal growth is vegetative production of numerous ramets that remain physically connected by stolon or rhizome internodes for a variable period of time. Clonal species can therefore develop into large interconnected structures consisting of an undetermined number of ramets that can quickly expand horizontally and colonize the surrounding area efficiently. Therefore, clonal growth was a common trait for many aggressive invasive plants (Liu *et al.*, 2006).

1-3 Studies of invasive plants in Taiwan

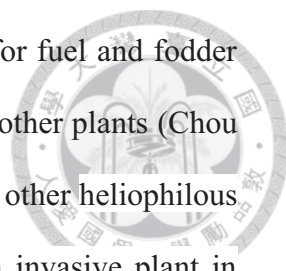
There are more than 2600 introduced species in Taiwan and among them about 600 species have become naturalized (Chiang *et al.*, 2003; Wu *et al.*, 2010). The introduction of plants mainly accompanied the settlements or immigrants to Taiwan started from about 300-400 years ago, and most of these introductions were for farming (Wu *et al.*, 2004). Chiang *et al.* (2003) listed 20 invasive plants in Taiwan: *Brachiaria mutica* (Forssk.) Stapf, *Cynodon plectostachyum* (Schum.) Pilger.,



Panicum maximum Jacq., *Pennisetum polystachion* (L.) Schult., *Pennisetum purpureum* Schumach., *Leucaena leucocephala* (Lam.) de Wit, *Mimosa diplotricha* C. Wright ex Sauvalle, *Sesbania cannabiana* (Retz.) Poir, *Eichhornia crassipes* (Mart.) Solms, *Lantana camara* L., *Alternanthera philoxeroides* (Mart) Griseb., *Amaranthus patulus* Bertoloni, *Ambrosia artemisiifolia* L., *Aster subulatus* Michaux var. *subulatus*, *Bidens pilosa* L. var. *radiata* Sch., *Chromolaena odorata* (L.) R. M. King & H. Rob., *Mikania micrantha* Kunth, *Parthenium hysterophorus* L., *Pluchea carolinensis* (Jacq.) G. Don, and *Pluchea sagittalis* (Lam.) Cabera. Most of these originated from Central America, and *Mikania micrantha*, *Leucaena leucocephala*, *Panicum maximum* and *Bidens pilosa* var. *radiata* have been studied.

Among these 20 invasive plants, *Mikania micrantha* Kunth (小花蔓澤蘭) (Asteraceae) has received the most attention because the species has threatened the native forest vegetation. It could climb on the plants, reduce their photosynthesis and cause severe economic damage and serious ecological problems (Hwang *et al.*, 2003). The study of *Mikania micrantha* revealed that it is a heliophilous species, flowers in November to December and produce about 170 thousand seeds per meter square (Kuo *et al.*, 2002). The researchers found that cutting the shoots near ground once a month and continuing for three month could reduce *Mikania micrantha* to less than 10%, and suggested that this might be a good way to manage it (Kuo *et al.*, 2002).

Leucaena leucocephala (銀合歡) is another invasive plant being studied in Taiwan. *Leucaena leucocephala* exhibits two growth forms: shrub-like Hawaiian type was introduced in Taiwan by Dutch in 1645 and the tree-like Salvador type was introduced by Taiwan's government in the 1970s for the purpose of afforestation and papermaking (Chiou *et al.*, 2013). However, with the improving economic conditions




in Taiwan since the 1980s, cultivation of *Leucaena leucocephala* for fuel and fodder has decreased drastically. However, due to its allelopathy effect on other plants (Chou and Kuo, 1986; Chai *et al.*, 2013), *Leucaena leucocephala* replaced other heliophilous species and formed pure forests in many habitats and become an invasive plant in Taiwan. It was suggested that injection herbicide into individual of *Leucaena leucocephala* and planting native tree at the same time might be a useful way to control and reduce *Leucaena leucocephala* populations (Wang and Hung, 2005).

Ho (2009) studied factors contributing to the successful invasion of *Panicum maximum* (大黍) into Dadu grassland in Taichung. The author found that *Panicum maximum* can resprout, grow faster and then produce more litters than *Miscanthus sinensis* in wet season. And high litter of *Panicum maximum* would induce fire more easily than that of *Miscanthus sinensis* during dry season. The burning events create new space for *Panicum maximum* to invade again. With the advantage of the morphological and photosynthetic traits in combination with the cycle of long wet season and short dry season with recurrent burning in Dadu mountain area, *Panicum maximum* gradually replace *Miscanthus sinensis*, and become the most dominant plant in Dadu Mountain area (Ho, 2009).

Bidens pilosa var. *radiata* (大花咸豐草) was first reported in Taiwan in 1984 (Peng *et al.*, 1998), and has become one of the most aggressive weeds in the past three decades (Wu *et al.*, 2004; Wu *et al.*, 2010). However, the plant receives less attention than *Mikania micrantha* and *Leucaena leucocephala*. Thus, this thesis focuses on studying this invasive plant.

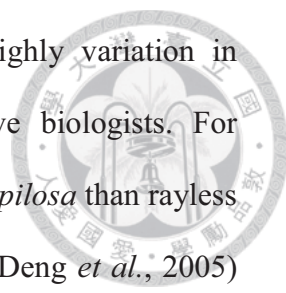
1-4 *Bidens pilosa* var. *radiata* and its congeners



There are about 240 species of *Bidens* (Asteraceae) in the world, most of them are in North and South America, and four species were listed in The Flora of Taiwan. They are *B. bipinnata*, *B. biternata*, *B. pilosa* and *B. tripartita*. According to the morphological characters, Sherff (1937) classified *B. pilosa* into six varieties: var. *pilosa*, var. *minor*, var. *radiata*, var. *bimucronata*, var. *calcicola*, and var. *alausensis*. Three of them were found in Taiwan, var. *minor*, var. *pilosa* and var. *radiata*. The most obvious differences in morphology among these three varieties are their flowers. The capitula of var. *pilosa* are discoid (without ray florets), and the capitula of var. *minor* and var. *radiata* are radiate (with ray florets). All these three varieties have 19 – 61 yellow disc florets, which are actinomorphic, hermaphroditic and incompletely protandrous (Huang and Kao, 2014). Only var. *minor* and var. *radiata* have 5-8 ray florets, which are zygomorphic and without androecium. However, the size of their ray florets is different. The ray florets of var. *radiata* are often longer than 10 mm, while the ray florets of var. *minor* are often shorter than 8 mm (Peng *et al.*, 1998).

In addition to the flower morphology, many other characters in these taxa are highly variable. For example, plants may be erect or decumbent; leaflets may be simple, tripartite, or dissected; and the achenes may have 2-4 awns. The highly variation in the morphology of *B. pilosa* interested botanists. Ballard (1986) studied 200 natural populations of *B. pilosa* that occurred in southern United States, Mexico, and Central America and found that they had three karyotypes: diploid, tetraploid and hexaploid (Ballard, 1986). Similar results were also found in Brazil populations (Grombone-Guaratini *et al.*, 2006; Maria *et al.*, 2008).

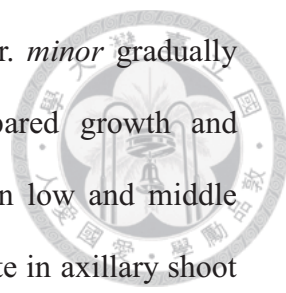
Pollinated by insects, the most frequent visitors to *Bidens* are hymenopterans and



lepidopterans (Grombone-Guaratini *et al.*, 2004), and having highly variation in flower morphology, *B. pilosa* also interested plant reproductive biologists. For example, it was found that outcrossing rate were higher in rayed *B. pilosa* than rayless one (Sun and Ganders, 1990). Furthermore, the genetic variation (Deng *et al.*, 2005) and genetic diversity (Tsai *et al.*, 2007) of *B. pilosa* in Taiwan were investigated. Heteromorphism were also found among achenes of *B. pilosa* on one capitulum, and which could affect the dispersal of achene (Rocha, 1966). The taxa in *Bidens* are a traditional medicine. Therefore, its chemical composition and function were also studied (Wang *et al.*, 1997; Alvarez *et al.*, 1999; Deba *et al.*, 2008).

Recently, *B. pilosa* var. *radiata* is considered as one of the serious invasive plants in Taiwan. Therefore, the advantage of var. *radiata* interests ecologists. Hsu (2006) found that a population of *B. bipinnata* was gradually displaced by *B. pilosa* var. *radiata* in an abandoned farm in South Taiwan. To understand how *B. pilosa* var. *radiata* replaced *B. bipinnata*, Hsu compared the growth and physiological performance between *B. pilosa* var. *radiata* and *B. bipinnata*. In growth and biomass allocation, she found that *B. pilosa* var. *radiata* had significantly higher root biomass ratio (RMR = root dry biomass / total plant dry biomass) than *B. bipinnata*. In addition, axillary shoots were only found in *B. pilosa* var. *radiata* but not observed in *B. bipinnata*. Therefore, the researchers concluded that higher root biomass ratio and having axillary shoots are important traits for the invasion of *B. pilosa* var. *radiata* (Hsu and Kao, 2014).

In addition to their difference in distribution range, *B. pilosa* var. *radiata* and var. *minor* have different population size at different altitudes. Field observation reveals that var. *radiata* is more widely distributed than var. *minor* at lowland. However,



population size of var. *radiata* gradually decreases but that of var. *minor* gradually increases with the increase of elevation. Huang (2008) compared growth and physiological performance between var. *radiata* and var. *minor* in low and middle elevation, and found that var. *radiata* had higher relative growth rate in axillary shoot and longer life span than var. *minor* when growing in lowland. Furthermore, low temperature significantly reduced the growth of adventitious roots and the percentage of achene germination in var. *radiata*. This result suggested that low temperature might be an important factor limiting the invasion of var. *radiata* into middle and high elevations of Taiwan.

1-5 Objectives and studies of the thesis

B. pilosa var. *minor* and var. *pilosa* have many traits similar to var. *radiata* but not found in *B. bipinnata*. For example, both varieties are capable of growing axillary shoots and adventitious roots (Huang, 2008). In addition, it was reported that var. *minor* and var. *pilosa* have wider distribution range than the other varieties in Central America (Ballard, 1986). Thirty years ago, *B. pilosa* var. *minor* widely distributed in Taiwan. However, after var. *radiata* being introduced into Taiwan, var. *radiata* has become a serious invasive plant and dominant over the other two varieties in Taiwan lowland. So, what traits make var. *radiata* become dominant over var. *minor* and var. *pilosa* in Taiwan lowland after its introduction? This is the question that I would like to ask in this study. And I hypothesized that the var. *radiata* could grow faster, produce more offspring, and spread faster than var. *minor* and var. *pilosa*.

To test the hypothesis, I compared growth, reproduction, and life history traits of var. *radiata* and the other two varieties to identify what biological traits make var.

radiata become dominant over the other two varieties and become invasive plant in Taiwan lowland. Results are presented in Chapter 2, 3, 4 and 5 of the thesis.

In Chapter 2, I compared the growth and morphology of these three varieties. I planted these three varieties, measured their growth, biomass and shoot architecture to understand whether var. *radiata* grows faster than the other two varieties.

In Hsu (2006), Huang (2008) and my pilot experiments conducted in a greenhouse, var. *radiata* did not set achenes in insects excluded greenhouses but *B. bipinnata* and *B. pilosa* var. *minor* and var. *pilosa* set numerous viable achenes. Therefore, I compared the reproduction biology of the three varieties of *B. pilosa*. In Chapter 3, the floral biology *B. pilosa* var. *radiata* was investigated. The secondary pollen presentation and dichogamy were the mechanism which often found in Asteraceae to preventing selfing. Therefore, I described the process of secondary pollen presentation and investigated the pollen viability and stigma receptivity of var. *radiata*. The floral morphology and P/O ratio of var. *radiata* were also reported in Chapter 3. This study has been published in *Botanical Studies* (Huang *et al.*, 2012).

Secondary pollen presentation and incomplete protandry were also observed in var. *minor* and var. *pilosa*. Therefore, these two phenomena were not the mechanisms contributing to no-achene production in var. *radiata* grown in greenhouses. In Chapter 4, I compared breeding system of the three varieties. Bagging experiments and pollen-pistil interactions were conducted. Result of this study was published in *Weed research* (Huang and Kao, 2014).

In addition to the breeding system, total achene production and life history traits also play an important role in affecting plants' dispersal and population size. In

Chapter 5, I compared the total achene production and life history traits of these three varieties.

A study on *Bidens pilosa* complex in Central America revealed that species with different polyploidy have different breeding systems. Could the three varieties also have different karyotypes? To answer this question, I collected samples of the three varieties from different regions of Taiwan and counted their chromosomal number. I also conducted crossing trials between invasive and non-invasive varieties. Results are presented in Chapter 6.

Final conclusions were drawn at the end of this thesis.



Chapter 2

A comparison of growth and morphology of the three varieties of *Bidens pilosa*, var. *minor*, var. *pilosa* and var. *radiata*, in two seasons

摘要

為了了解為何大花咸豐草能快速地佔領某一入侵地，本研究分別在夏季與秋季栽植這三個變種，比較其生長、生物量分配與葉部特徵，以期找出讓大花咸豐草比其它兩變種佔優勢的生物特徵。

結果顯示：三者夏季的相對生長速率比秋季高，而與小白花鬼針和白花鬼針比起來，大花咸豐草在夏季所累積的生物量顯著較高，但在秋季，三者所累積的生物量則無顯著差異。不管在夏季或秋季，大花咸豐草並沒有顯著較高的地上部/地下部生物量的比例、葉面積比或比葉面積。但大花咸豐草於夏季有顯著較多的側枝生物量比例。據此推測：具較多的側枝生物量比例，可以增加光的截取，此可能是使得大花咸豐草於夏季具生長優勢的原因。此外，雖然三變種的枝條均會長不定根，但大花咸豐草的不定根生長速率較另外兩變種快，且其最接近地面的側枝生長角度顯著低於其它兩變種，最有可能會接觸到地面，進而長出不定根。因此，推測大花咸豐草藉由側枝進行無性繁殖生長的潛力應大於其它兩變種。（此推測在本論文之第五章獲得證實。）

大花咸豐草在夏季累積較多的生物量，並有較好的無性繁殖生長潛力，配合臺灣溫暖高光的熱帶、亞熱帶氣候，可能是造成大花咸豐草比其它兩變種更適合在臺灣生長，且成為入侵植物的原因之一。

關鍵詞：咸豐草(*Bidens pilosa*)、生物量分配、無性繁殖生長、入侵植物、葉部特徵、枝條分配

Abstract

To identify traits making *B. pilosa* var. *radiata* spreading faster than the other two varieties in a certain habitat, I cultured these three varieties in summer and fall, compared their growth and traits related to growth, and tested the hypothesis that var. *radiata* grows faster than the other two varieties.

All three varieties accumulated more biomass in summer than in fall and var. *radiata* grew faster and accumulated more biomass than the other two varieties in summer. In the morphological traits, var. *radiata* did not have higher shoot/root ratio, leaf area ratio or specific leaf area both in summer and in fall. However, var. *radiata* had more biomass allocating to axillary shoots in summer which could increase light interception for photosynthesis and hence enhance growth. In addition, although the three varieties were capable of growing adventitious roots from shoots, the adventitious roots of var. *radiata* grew faster than the other two varieties, and the branching angle of the axillary shoots growing from the first node of main stem was significantly more horizontal in var. *radiata* than in the other two varieties. The more horizontally-oriented axillary shoots had higher chance to touch the ground and grow adventitious roots. More axillary shoots, which are also more horizontally oriented, also provides var. *radiata* more opportunity for clonal growth than the other two varieties. (This speculation was confirmed in Chapter 5 of this thesis.)

Comparing to the other two varieties, var. *radiata* accumulating more biomass in summer and having better vegetative reproduction potential in combination of warm and high light climate in Taiwan might be one of the reasons making var. *radiata* more dominant than the other two varieties and becoming invasive in Taiwan.

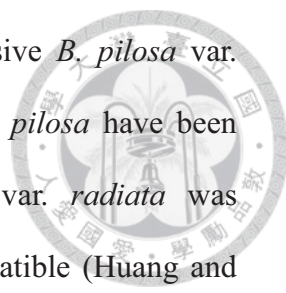
Key words: *Bidens pilosa*, Biomass allocation, Clonal growth, Invasive plant, Leaf character, Shoot allocation



Introduction

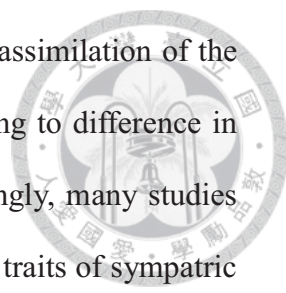
Invasive species often cause harmful impacts to biodiversity of some ecosystem (Begon *et al.*, 1996), and affect regional economics and society, thus, the invasive biology has become an important subject not only in ecological study but also in economy (Pimentel *et al.*, 2000). Successful invaders often have some traits promoting their invasiveness (van Kleunen *et al.*, 2010). Identifying these traits might help us finding ways to control and manage the invasive species and identifying potential invaders. Accordingly, effective action could be taken to reduce their negative effect.

Bidens pilosa L. var. *radiata* Sch. Bip., an annual or perennial herb belonging to Asteraceae, was first collected and documented in 1937 (Sherff, 1937) and recorded in Taiwan in 1984 (Peng *et al.*, 1998; Wu *et al.*, 2004). The objective of the study is to understand what make *B. pilosa* var. *radiata* become an invasive plant in Taiwan. Phylogenetically related plants may share more common traits and require more overlapping resource than unrelated plants (Goldberg, 1987; Rauschert and Shea, 2012). Thus, comparing traits between related invasive and non-invasive plants might be more informative in understanding what makes plants invasive. This approach has been used in many studies (McDowell, 2002; Feng and Fu, 2008; Feng *et al.*, 2008). Two additional varieties of *B. pilosa*, var. *minor* and var. *pilosa*, are found in Taiwan (Peng *et al.*, 1998). Though var. *radiata* was introduced into Taiwan later than var. *minor* and var. *pilosa*, within the 30 years, var. *radiata* has become a serious invasive plant and dominant over the other two varieties in Taiwan lowland. Therefore, in this study, I compared the var. *radiata* and the other two varieties, var. *minor* and var. *pilosa*, to identify what traits making var. *radiata* more dominant than the other two



varieties in Taiwan lowland now. Breeding systems of the invasive *B. pilosa* var. *radiata* and non-invasive *B. pilosa* var. *minor* and *B. pilosa* var. *pilosa* have been investigated, and it was found that the invasive *B. pilosa* var. *radiata* was self-incompatible while the non-invasive varieties were self-compatible (Huang and Kao, 2014). In addition to the difference in breeding system, vegetative and life history traits might also contribute to the invasiveness (Zheng *et al.*, 2009; Ordonez *et al.*, 2010; Scharfy *et al.*, 2011). Thus, in this study I investigated vegetative aspects, including growth rate, biomass allocation, plant architectures and leaf characteristics of these three varieties.

Growth is one of the most relevant traits determining plant performance in the field. Plants have high growth rate would outcompete other sympatric species in resources uptake and become dominant. Many invasive plants were found growing faster and accumulating more biomass than non-invasive plants (Zheng *et al.*, 2009; van Kleunen *et al.*, 2010). Accordingly, traits related to growth were also important in governing the invasiveness. For example, biomass allocation plays an important role in determining relative growth rate (RGR). It has been found that plants allocating more biomass to above ground components (have higher shoot/root ratio) might have a higher RGR than those allocating more biomass to roots (Wagg *et al.*, 2011; Sorrell *et al.*, 2012). In all above ground components, leaf is the most important organ to assimilate carbon to accumulate biomass. Therefore, specific leaf area (SLA = leaf area / leaf dry biomass) is an extremely important trait in the regulation and control of plant functions such as carbon assimilation and carbon allocation. Plants with higher SLA would have larger assimilatory surfaces and hence increase the capacity to assimilate CO₂ for a given amount of resources invested in photosynthetic tissues (Lambers and Poorter, 1992; Baruch and Goldstein, 1999). Leaf area ratio (LAR =



total leaf area / total plant dry biomass) is another trait affecting C assimilation of the whole plant. Thus, these two characters are major traits contributing to difference in plant growth (Westoby *et al.*, 2002; Wright *et al.*, 2004). Accordingly, many studies have found significant differences between the aforementioned leaf traits of sympatric invasive and native species, with invasive species having higher LAR or having higher SLA than non-invasive plants (Pattison *et al.*, 1998; Baruch and Goldstein, 1999; Smith and Knapp, 2001; Hamilton *et al.*, 2005; Leishman *et al.*, 2007; Ordonez *et al.*, 2010; Osunkoya *et al.*, 2010; Scharfy *et al.*, 2011).

In a preliminary experiment, I observed that the three varieties of *B. pilosa* had different plant architecture of shoots. Plant architecture, including the degree of branch and branch angle, would affect light interception and hence might also play an important role in regulation plant growth. After comparing *B. bipinnata* and *B. pilosa* var. *radiata*, Hsu (2006) found that only *B. pilosa* var. *radiata* grew adventitious root from stem segments and suggested that the invasive plant might be able to reproduce vegetatively. Huang (2008) reported that *B. pilosa* var. *radiata* had higher relative growth rate of axillary shoots and better growth of adventitious roots than var. *minor*. Thus, the potential for clonal growth might also contribute to the dominance of *B. pilosa* var. *radiata*.

In this study I compared vegetative growth, pattern of biomass allocation, leaf characters (LAR and SLA) and plant architecture among plants of the three varieties cultured in summer and fall. I tested the hypothesis that in comparison to the other two varieties, the invasive varieties *B. pilosa* var. *radiata* allocates more biomass to shoot, and has higher LAR and SLA thus grows faster and accumulates more biomass during growth period. I also evaluated the clonal growth potential among the three

varieties by comparing their plant architecture of *B. pilosa* in Taiwan.



Materials and Methods

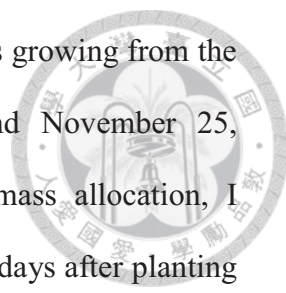
Plant materials

Bidens pilosa (Asteraceae) is an annual or perennial herb with erect stems and opposite leaves. The three varieties of *B. pilosa*, *B. pilosa* var. *minor* (abbreviated as var. *minor*), *B. pilosa* var. *pilosa* (abbreviated as var. *pilosa*) and *B. pilosa* var. *radiata* (abbreviated as var. *radiata*), are distributed on roadsides and cultivated fields from low to middle elevations in Taiwan (Peng *et al.*, 1998). They can be distinguished by their florets. All three varieties have disc florets (No. 19 - 77), but only var. *radiata* and var. *minor* have ray florets (No. 5 - 8) on each capitulum. Ray florets of var. *radiata* are usually longer than 10 mm, but those of var. *minor* are shorter than 8 mm (Peng *et al.*, 1998).

Seedlings germinated from achenes collected from central Taiwan (23°26' N, 120°36' E, 500 m a.s.l.) where the three varieties growing sympatrically were transplanted into 4L (18.5 cm×19.5 cm) pots and placed in a greenhouse of National Taiwan University. Six plants for each variety were planted in summer for 56 days (from 16th June to 11th August) and in fall for 70 days (from 25th September to 4th December) of 2009, respectively.

Vegetative growth, biomass allocation and leaf characters

Stem heights and node numbers were measured twice a week after plants



producing one to three pairs of leaves. The angles of axillary shoots growing from the lowest node of main stem were measured on August 11 and November 25, respectively. To avoid the effect of achene production on biomass allocation, I harvested plants before they set achene, which was 56 days and 70 days after planting for the summer and fall experiments, respectively. Plants were dissected into roots, shoots and reproductive organ components. Shoots were further separated into main stems and axillary shoots. Leaves growing on main stems and on axillary shoots were excised and their leaf area was measured with a leaf area meter (Li-3000, Li-Cor, Lincoln, Nebraska, USA) immediately after harvested. Each plant component was dried in an oven at 80°C over 48 hrs and then weighted. Total biomass, the fraction of biomass of each component and leaf area ratio ($LAR = \text{total leaf area} / \text{total dry biomass}$) and the specific leaf area ($SLA = \text{leaf area} / \text{leaf dry weight}$) were calculated. The biomass allocating to main stems, primary and secondary axillary shoots were calculated as the ratio of biomass of each shoot component to total shoot biomass (the sum of main stems, primary and secondary axillary shoots).

Adventitious root

Shoots with fully expended leaves were excised from plants (with five to six nodes on main stem, $n = 10$) and immersed into flasks containing 400 ml water on 7th April, 2008. The ability of these shoots to grow adventitious roots was observed. Sixteen days after the treatment (22nd April, 2008), the produced roots were harvested, dried in an oven at 80°C over 48 hrs and then their dry weight were measured.

Statistic analysis

Two way analysis of variance (ANOVA) was conducted (general linear model procedure of SAS, release 9.1, SAS Inst. Inc., USA) to determine whether variables were significantly different between two growing seasons and among the three varieties. The difference of blooming date and adventitious roots among three varieties were analyzed by one way ANOVA. If the null hypothesis was rejected after the analysis of ANOVA, Student-Newman-Keuls test was used for multiple comparisons.

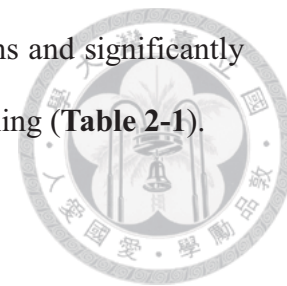
Results

Flowering

In summer, within 56 days of culturing period, all six plants of var. *minor* had flowers. The juvenile period of var. *minor* was 48.2 days. When var. *minor* started to flower, the plant height was about 60.5 cm with 10.8 nodes on the main stems (**Table 2-1**). The var. *pilosa* and var. *radiata* started to flower later than var. *minor*, only three individuals of var. *pilosa* and none of var. *radiata* had flowers within 56 days of culturing period in summer. The main stem (including node number and height) of these three varieties all stopped growing after blooming. Therefore, in var. *pilosa*, non blooming individuals ($n = 3$) were significantly higher ($F_{1,4} = 24.72$; $P < 0.01$) and with more nodes ($F_{1,4} = 39.20$; $P < 0.01$) on main stems than blooming ones ($n = 3$) (**Table 2-1**).

In fall, after 70 days of growing period, all cultured individuals of these three varieties had flowers. The juvenile period of var. *radiata* was longer than var. *minor*

and var. *pilosa*. Furthermore, var. *radiata* had the tallest main stems and significantly more nodes on main stems than the other two varieties when blooming (**Table 2-1**).

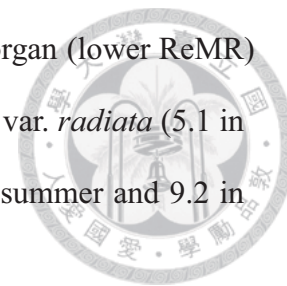


Vegetative growth and biomass allocation

The results of RGR, biomass accumulation, biomass allocation, shoot/root ratio and LAR of three varieties growing in summer and fall are shown in **Table 2-2**. The RGR_{DW} of plants growing in summer were 191.8 - 215.2 mg day⁻¹, which were significantly higher than those growing in fall (128.4 - 142.0 mg day⁻¹) (**Table 2-3**). Cultured in summer, var. *radiata* accumulated 12.1 g dry mass in cultured period, this is significantly higher than var. *minor* (10.9 g) and var. *pilosa* (10.7 g) ($F_{2, 15} = 3.74$; $P < 0.05$). However, the biomass accumulations in fall were not significantly different among the three varieties ($F_{2, 15} = 0.45$; $P = 0.64$).

A similar pattern of biomass allocation to vegetative components was found among the three varieties in both seasons. The most biomass was allocated to stems (> 50% in summer and 46.2% - 47.9% in fall), the least to roots (12.9% - 16.6% in summer and 9.9% - 16.6% in fall), and the intermediate to leaves (27.1% - 32.1% in summer and 29.3% - 32.2% in fall) (**Table 2-2**). The result of two-way ANOVA showed that the SMR, ReMR and shoot/root ratio of plants growing in summer significantly differed to those of plants growing in fall (**Table 2-3**). The SMR of all three varieties were higher in summer than in fall. On the contrast, the ReMR and shoot/root ratio of all three varieties were higher in fall than in summer (**Table 2-2**). The result of two-way ANOVA showed that the RMR, LMR, ReMR and shoot/root ratio of plants were significantly different among the three varieties (**Table 2-3**). In comparison to the other two varieties, var. *radiata* allocated proportionally more

biomass to root (a higher RMR) and less biomass to reproductive organ (lower ReMR) in both experiments (**Table 2-2**). Therefore, the shoot/root ratios of var. *radiata* (5.1 in summer and 5.3 in fall) were less than those of var. *minor* (6.9 in summer and 9.2 in fall) and var. *pilosa* (6.9 in summer and 7.6 in fall) (**Table 2-2**).



Significant difference in LAR was found among the three varieties and between plants growing in summer and in fall (**Table 2-3**). *B. pilosa* var. *minor* had higher LAR than the other two varieties (**Table 2-2**), and all three varieties had significant increase in LAR in fall than in summer.

Shoot allocation

In both seasons, all three varieties had primary axillary shoots but only *B. pilosa* var. *radiata* grew secondary axillary shoots (**Table 2-4**). Different pattern of biomass allocation to main stems and axillary shoots was found among the three varieties cultured in summer (**Table 2-4**). In summer, var. *radiata* allocated similar biomass to main stems and axillary shoots (primary + secondary axillary shoots), but var. *minor* and var. *pilosa* allocated significantly more biomass to main stems than to axillary shoots. The difference in biomass allocation to main stems and to axillary shoots among the three varieties in summer was not found in fall. All three varieties cultured in fall allocated significantly more biomass to axillary shoots than to main stems. After analyzed with two-way ANOVA, it was found that the biomass allocation to main stems and secondary axillary shoots were significantly different between two growing seasons, among three varieties and had significant interaction between seasons and varieties (**Table 2-5**). However, biomass allocation to primary axillary shoots only differed significantly between two seasons and had significant interaction

between seasons and varieties (**Table 2-5**).

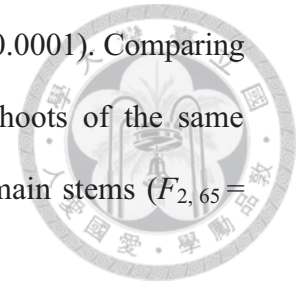
In both seasons, the branching angles of axillary shoots growing from the lowest nodes of main stems were significantly lower in var. *radiata* than in the other two varieties (summer: $F_{2,15} = 11.47$, $P < 0.001$; fall: $F_{2,15} = 65.60$, $P < 0.0001$) (**Figure 2-1**).

Leaf characters

Comparing plants of all three varieties growing in the same season, the three varieties had similar total leaf area ($F_{2,30} = 1.86$, $P = 0.17$) (**Figure 2-2 A - C**). Comparing two seasons, all three varieties had significantly increased in total leaf area in fall ($F_{1,30} = 8.87$, $P < 0.01$) (**Figure 2-2 A - C**). Comparing leaf area allocating to main stems and to axillary shoots, more leaves were found on axillary shoots than on main stems in var. *radiata* in both seasons (**Figure 2-2A**). However, different pattern was found in the two non-invasive varieties. In var. *minor* and var. *pilosa*, more leaves were found on main stems than on axillary shoots in summer (**Figure 2-2 B and C**). But in fall, var. *minor* and var. *pilosa* had significantly more leaves on axillary shoots than on main stems (**Figure 2-2 B and C**). Furthermore, because only var. *radiata* had secondary axillary shoots, the leaves on secondary axillary shoots were only found in var. *radiata*.

SLA had found significant difference between two seasons, among the three varieties and among leaf positions (**Figure 2-2 D - F**). For each variety, plants growing in summer had lower SLA than those in fall ($F_{1,65} = 211.31$; $P < 0.0001$). Comparing leaves on the same order of shoots among the three varieties, var. *radiata*

had lower SLA than var. *minor* and var. *pilosa* ($F_{2, 65} = 31.68$; $P < 0.0001$). Comparing the SLA between leaves growing on main stems and axillary shoots of the same variety, leaves on axillary shoots had higher SLA than those on main stems ($F_{2, 65} = 53.57$; $P < 0.0001$).



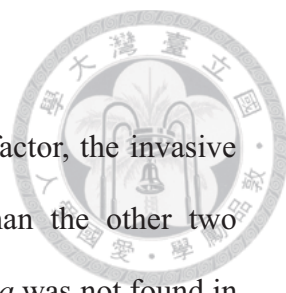
Adventitious root

The duration from shoots immersed into water to adventitious roots starting to sprout of var. *radiata* was shorter than those of var. *minor* and var. *pilosa* (**Table 2-6**). Shoot segments of var. *radiata* started to grow adventitious roots 3 - 4 days, those of var. *minor* 4 - 10 days and var. *pilosa* 4 - 8 days after being immersed into water (**Table 2-6**). After immersed into water for 10 days, all shoot segments grew adventitious roots. After immersed into water for 16 days, *B. pilosa* var. *radiata* grew significantly heavier adventitious roots than var. *minor* and var. *pilosa*. But no significant differences were found in the adventitious root numbers and total root lengths among the three varieties (**Table 2-6**).

Discussion

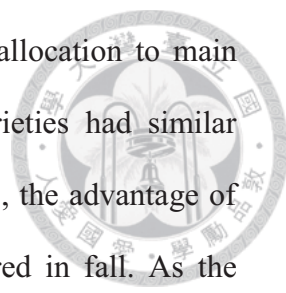
In comparison to summer, the growth of the three varieties was significantly reduced in fall (**Table 2-2**). This suggested that summer was a better growth season for these three varieties. In Taiwan, the light intensity and air temperature were higher in summer than in fall (Chang and Yang, 2003). Less biomass (**Table 2-2**), more leaf area and higher SLA (**Figure 2-2**) found in all three varieties cultured in fall than in summer suggested that light availability is a limiting factor for the growth of the three

varieties in fall.



Growing in summer when light availability is not a limiting factor, the invasive var. *radiata* did grow faster and accumulated more biomass than the other two varieties in summer. However, the advantage of invasive var. *radiata* was not found in fall. In general, plants having higher shoot/root ratio would grow faster (Wagg *et al.*, 2011; Sorrell *et al.*, 2012). The result that var. *radiata* had the lowest shoot/root ratio among the three varieties suggested that the ratio is not the main factor determining the difference in growth rate of the three varieties. LAR and SLA are also factors might affect plant growth (Westoby *et al.*, 2002; Wright *et al.*, 2004). However, in comparison to the other two varieties, *B. pilosa* var. *radiata* did not have higher LAR (**Table 2-2**) or SLA in summer (**Figure 2-2**). Thus, it is unlikely that difference in LAR or SLA contributes to the different growth rate among the three varieties. Another factor that might affect the growth rate is photosynthetic rate. However, in a preliminary experiment, I found that var. *radiata* did not have a higher photosynthetic rate than var. *pilosa* (unpublished data). Then, what could be the factor contributing to their difference in biomass accumulation in summer?

I suggested that differences found in the biomass allocation between main stems and axillary shoots among the three varieties might explain their difference in biomass accumulation in summer. In summer, var. *radiata* allocated significantly more biomass to axillary shoots (**Table 2-4**) and had more horizontal axillary shoots (**Figure 2-1**) than the other two varieties. The combination of allocating more biomass to axillary shoots and maintaining more horizontally oriented axillary shoots might help var. *radiata* increasing light interception, reducing self-shading hence growing faster and accumulating more biomass than the other two varieties in summer.

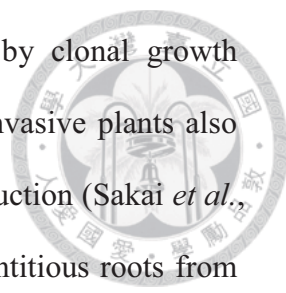


However, no significant difference in the proportion of biomass allocation to main stems and axillary shoots was found in fall when all three varieties had similar proportion of increased biomass allocation to axillary shoots. Thus, the advantage of increasing light interception of var. *radiata* in summer disappeared in fall. As the result, the three varieties accumulated similar biomass in fall.

In general, invasive plants have early reproductive age (Rejmánek and Richardson, 1996). However, in this study I found that var. *radiata* flowered later than the other two non-invasive varieties (**Table 2-1**).

The result that the growth superior of var. *radiata* to var. *minor* and var. *pilosa* was only found in summer but not in fall and var. *radiata* flowered significantly later than the other two varieties suggested that var. *radiata* might have more growth advantage than var. *minor* and var. *pilosa* in habitats with warm and high irradiation conditions. The distribution patterns of the three varieties in America and in Taiwan are consistent with the prediction from the growth experiment. In north America, var. *minor* and var. *pilosa* were the most invasive varieties (Ballard, 1986; Grombone-Guaratini *et al.*, 2004). On the contrary, var. *radiata* mainly distributed on coastal (Grombone-Guaratini *et al.*, 2004), moist tropical lowlands and eastern coast of Mexico (Ballard, 1986). While in Taiwan, var. *minor* and var. *pilosa* are found distributed mainly in middle elevations (personal observation), but var. *radiata* is an aggressive weed in lowland (Peng *et al.*, 1998; Huang, 2008).

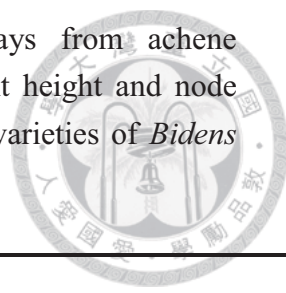
Plants with clonal growth ability could develop into large interconnected network, quickly expand horizontally and colonize the surrounding areas efficiently. Accordingly, many invasive species were found having clonal growth ability (Yu *et al.*, 2009). For example, *Spartina anglica*, one of the most widespread and locally



abundant invasive plants in North American, spreads in local by clonal growth (Thompson, 1991). A review focusing on population biology of invasive plants also suggested that invasiveness was highly related to vegetative reproduction (Sakai *et al.*, 2001). In this study, I found that all three varieties can grow adventitious roots from shoots indicating their potential of vegetative reproduction. However, among the three varieties, only var. *radiata* has secondary axillary shoots. Furthermore, var. *radiata* had more horizontal angles in the lowest axillary shoots than the other two varieties. The more horizontal oriented axillary shoots would have better chance to touch the ground than vertically oriented axillary shoots. When there is a chance for var. *radiata* to touch the ground, its primary axillary shoots could become runner and the secondary axillary shoots then turn into ramets. These results suggested that var. *radiata* has better potential to increase population size by clonal growth. Accordingly, the architecture of var. *radiata* might play an important role in helping the invasive variety increasing population size.

In comparison to the other two varieties, var. *radiata* accumulated significantly more biomass in summer but not in fall. Differences in biomass allocation between main stems and axillary shoots among the three varieties in the two seasons might explain the phenomenon. Differences in plant architecture between var. *radiata* and the other two varieties suggested that var. *radiata* has better clonal growth potential than var. *minor* and var. *pilosa*. The combination of these growth traits and the growth environment of Taiwan render var. *radiata* invasive in Taiwan.

Table 2-1 Blooming of three varieties. Juvenile period (days from achene germinating to first flower blooming of a plant), plant height and node number on main stems (means \pm SE, $n = 6$) of three varieties of *Bidens pilosa* cultured in summer and in fall of 2009.



Blooming parameter	Variety			P
	var. <i>radiata</i>	var. <i>minor</i>	var. <i>pilosa</i>	
Summer				
Juvenile period (days)	N. A.	48.2 \pm 1.0	47.3 \pm 3.0 [†]	-
Plant height (cm)	(67.6 \pm 5.5*)	60.5 \pm 2.7	61.3 \pm 3.9 (85.3 \pm 2.9*)	-
Node number	(12.3 \pm 0.4*)	10.8 \pm 0.5	10.7 \pm 0.7 (15.3 \pm 0.3*)	-
Fall				
Juvenile period (days)	59.8 \pm 3.4 ^a	49.2 \pm 0.3 ^b	50.2 \pm 0.6 ^b	< 0.01
Plant height (cm)	49.5 \pm 5.7 ^a	34.5 \pm 0.6 ^b	43.0 \pm 2.8 ^{ab}	0.04
Node number	8.7 \pm 0.5 ^a	7.0 \pm 0.0 ^b	7.7 \pm 0.2 ^b	< 0.01

N. A.: Not available.

*: data were calculated from non-blooming plants.

[†]: Because only three plants blooming before harvested, the means and SE were calculated from three blooming individuals.

Means followed by different letters differed significantly among the three varieties ($P < 0.05$).

Table 2-2 Growth, biomass allocation and LAR of three varieties. Relative growth rate of shoot height (RGR_{SH}), relative growth rate of dry weight (RGR_{DW}), total biomass, proportion of biomass allocation to roots (RMR), stem (SMR), leaf (LMR) and to reproduction (ReMR), the ratio of above ground component verse root (shoot/root ratio) and the ratio of total leaf area to total plant dry biomass (LAR) (means \pm SE, $n = 6$) of three varieties of *Bidens pilosa* cultured in summer and in fall of 2009.

Variable	Variety		
	var. <i>radiata</i>	var. <i>minor</i>	var. <i>pilosa</i>
Summer			
RGR _{SH} (mm day ⁻¹)	12.1 \pm 1.0	12.2 \pm 0.6	13.5 \pm 0.9
RGR _{DW} (mg day ⁻¹)	215.2 \pm 6.7	195.2 \pm 7.3	191.8 \pm 5.5
total biomass (g)	12.1 \pm 0.4	10.9 \pm 0.4	10.7 \pm 0.3
RMR (%)	16.6 \pm 1.0	12.9 \pm 0.7	13.0 \pm 0.9
SMR (%)	51.2 \pm 1.8	52.3 \pm 0.8	57.5 \pm 2.4
LMR (%)	32.1 \pm 1.3	31.1 \pm 0.9	27.1 \pm 1.8
ReMR (%)	0.05 \pm 0.04	3.7 \pm 0.5	2.4 \pm 1.2
Shoot/Root ratio	5.1 \pm 0.4	6.9 \pm 0.4	6.9 \pm 0.7
LAR (cm ² g ⁻¹)	139.1 \pm 4.1	168.5 \pm 5.5	137.1 \pm 9.2
Fall			
RGR _{SH} (mm day ⁻¹)	7.3 \pm 0.8	5.1 \pm 0.1	6.6 \pm 0.5
RGR _{DW} (mg day ⁻¹)	139.2 \pm 13.0	128.4 \pm 8.9	142.0 \pm 9.4
total biomass (g)	9.7 \pm 0.9	9.0 \pm 0.6	9.9 \pm 0.7
RMR (%)	16.6 \pm 1.7	9.9 \pm 0.5	11.8 \pm 0.7
SMR (%)	46.2 \pm 2.1	47.9 \pm 1.3	46.7 \pm 1.6
LMR (%)	32.2 \pm 1.9	31.8 \pm 1.2	29.3 \pm 1.2
ReMR (%)	5.0 \pm 1.2	10.4 \pm 1.1	12.2 \pm 1.2
Shoot/Root ratio	5.3 \pm 0.5	9.2 \pm 0.6	7.6 \pm 0.5
LAR (cm ² g ⁻¹)	193.9 \pm 8.5	227.0 \pm 12.2	198.7 \pm 10.5

Table 2-3 Effect of season and variety on growth, biomass allocation and LAR.

Results of two-way ANOVA for the effect of seasons (summer or fall), varieties (three varieties) and their interaction on RGR_{SH} , RGR_{DW} , total biomass, proportion of biomass allocation to roots (RMR), stem (SMR), leaf (LMR) and to reproduction (ReMR), shoot/root ratio and the ratio of total leaf area to total plant dry biomass (LAR).

Variable	Season ($df=1$)		Varieties ($df=2$)		Season \times Varieties ($df=2$)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
RGR_{SH} (mm day ⁻¹)	116.20	***	1.99	ns	1.67	ns
RGR_{DW} (mg day ⁻¹)	79.80	***	1.58	ns	1.14	ns
total biomass (g)	12.45	**	1.29	ns	0.90	ns
RMR (%)	2.92	ns	15.69	***	1.14	ns
SMR (%)	22.42	***	1.92	ns	2.03	ns
LMR (%)	0.68	ns	4.26	*	0.27	ns
ReMR (%)	75.88	***	14.41	***	3.08	ns
Shoot/Root ratio	5.74	*	15.08	***	2.34	ns
LAR (cm ² g ⁻¹)	65.87	***	8.04	**	0.08	ns

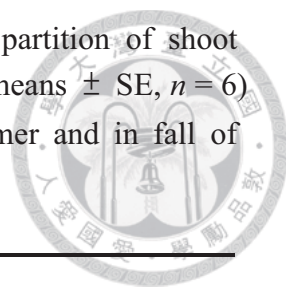
ns: significance level are $P > 0.05$ (non-significant).

*: $P < 0.05$.

**: $P < 0.01$.

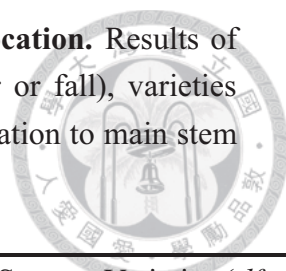
***: $P < 0.001$.

Table 2-4 Shoot biomass allocation of three varieties. The partition of shoot biomass allocating to main stem and axillary shoots (means \pm SE, $n = 6$) of three varieties of *Bidens pilosa* cultured in summer and in fall of 2009.



Variable	Variety		
	<i>var. radiata</i>	<i>var. minor</i>	<i>var. pilosa</i>
Summer			
Main stem (%)	48.6 \pm 2.3	64.8 \pm 3.4	64.3 \pm 4.5
Primary axillary shoot (%)	50.6 \pm 2.2	35.2 \pm 3.4	35.7 \pm 4.5
Secondary axillary shoot (%)	0.8 \pm 0.7	0	0
Fall			
Main stem (%)	28.8 \pm 4.8	29.8 \pm 2.0	28.8 \pm 1.1
Primary axillary shoot (%)	65.9 \pm 3.7	70.2 \pm 2.0	71.2 \pm 1.1
Secondary axillary shoot (%)	5.2 \pm 2.0	0	0

Table 2-5 Effect of season and variety on shoot biomass allocation. Results of two-way ANOVA for the effect of seasons (summer or fall), varieties (three varieties) and their interaction on biomass allocation to main stem and axillary shoot.



Variable	Season (<i>df</i> = 1)		Varieties (<i>df</i> = 2)		Season×Varieties (<i>df</i> = 2)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Main stem (%)	123.70	***	4.13	*	3.65	*
Primary axillary shoot (%)	133.70	***	1.98	ns	7.17	**
Secondary axillary shoot (%)	4.20	*	8.07	**	4.20	*

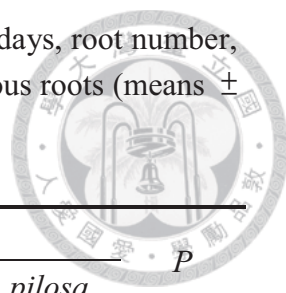
ns: significance level are $P > 0.05$ (non-significant).

*: $P < 0.05$.

** : $P < 0.01$.

***: $P < 0.001$.

Table 2-6 Growth of adventitious root of three varieties. The days, root number, total root length and total root dry weight of adventitious roots (means \pm SE, $n = 10$) of three varieties of *Bidens pilosa*.



Variable	Variety			<i>P</i>
	var. <i>radiata</i>	var. <i>minor</i>	var. <i>pilosa</i>	
Days	3.7 \pm 0.2 ^b	5.5 \pm 0.6 ^a	5.4 \pm 0.5 ^a	0.02
Root number	16.2 \pm 1.7	12.6 \pm 2.1	14.9 \pm 1.2	0.34
Total root length (cm)	241.2 \pm 25.4	148.2 \pm 25.0	198.2 \pm 31.8	0.07
Total root dry weight (mg)	31.2 \pm 2.0 ^a	17.3 \pm 2.4 ^b	17.2 \pm 2.5 ^b	0.0002

Days: duration from shoot immerse into water to adventitious roots start to sprout.

Root number: the total adventitious root number on 22nd April 2008.

Total root length: the total adventitious root length on 22nd April 2008.

Total root dry weight: the total adventitious root dry weight on 22nd April 2008.

Means followed by different letters differed significantly among the three varieties ($P < 0.05$).

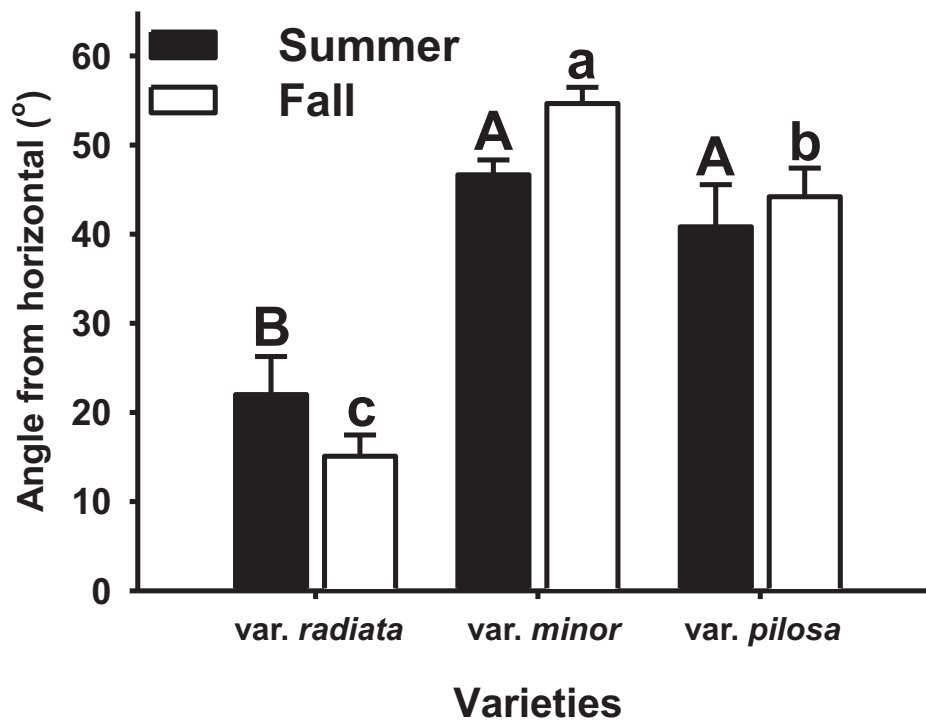


Figure 2-1 The angle of axillary shoots of three varieties. The angle from the horizontal (means \pm SE, $n = 6$) of axillary shoots growing from the lowest node three varieties of *Bidens pilosa* cultured in summer (black bar) and in fall (open bar) of 2009. Black bars with different capital letters represent significant difference ($P < 0.05$) being found among the three varieties in summer, and open bars with different lower cases represent significant difference ($P < 0.05$) among the three varieties in fall.

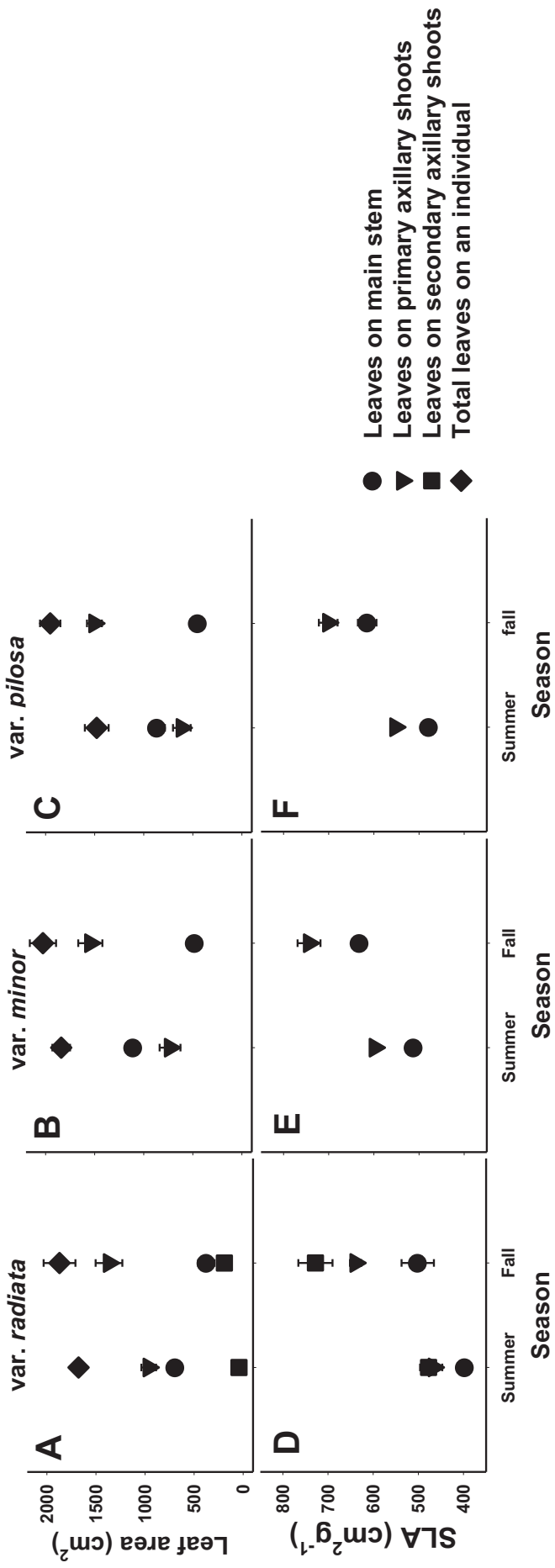


Figure 2-2 Leaf area and SLA of three varieties. Leaf area of total leaves on an individual (A, B, C), leaf area (A, B, C) and specific leaf area (SLA) (D, E, F) of leaves on main stem, primary axillary shoots and secondary axillary shoots (only found in var. *radiata*: $n = 3$ in summer and $n = 4$ in fall, respectively) of three varieties of *Bidens pilosa* cultured in summer and in fall of 2009.





Chapter 3

Floral biology of *Bidens pilosa* var. *radiata*

(This chapter has been published in *Botanical Studies* **53**: 501-507, 2012)

摘要

了解入侵植物的生殖生物學可以幫助我們有效控制其擴散。本章節主要在探討大花咸豐草的花部生物學，包含其（1）花部形態，（2）次級花粉呈現，（3）花粉/胚珠比。

將採自嘉義的大花咸豐草瘦果種植在盆子中並放置於台大實驗農場；待植物開花後，觀察和分析其特徵。觀察發現大花咸豐草的一個頭狀花序上，有5-8個舌狀花和許多管狀花；除了觀察管狀花、花藥筒、花柱與花粉的形態，並定量一個頭狀花序上管狀花的數量、一個管狀花中花粉的數量與花粉/胚珠比；又根據花藥筒與花柱的形態將小花綻放過程分為六個階段，並分別檢驗這六個階段的花粉與柱頭活性。結果發現：一個頭狀花序上管狀花的數量(19 - 61，平均44.1)與一個管狀花所含花粉數量(6556 - 11378)在個體間有顯著差異。大花咸豐草的次級花粉呈現機制是由花柱伸長將花粉推出花藥筒。花粉與柱頭活性測定結果顯示，雖然雄性功能成熟期較雌性成熟期早，但兩者並未完全分開，故為不完全雄先熟。又根據花粉/胚珠比(8827 ± 464)的結果推測大花咸豐草的有性繁殖方式可能是完全異交。

關鍵詞：自交、大花咸豐草、頭狀花序、花粉/胚珠比、雄先熟、次級花粉呈現、柱頭乳突、掃粉毛、異交

Abstract

Knowledge of reproductive biology of invasive plants is necessary in developing effective ways to control their spread. The objective of this study was to investigate the floral biology including; (1) floral structure, (2) the process of secondary pollen presentation, and (3) pollen/ovule ratio (P/O ratio) of this invasive plant.

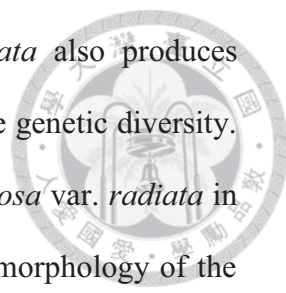
Plants were grown in pots from achenes and placed at the experimental farm of the National Taiwan University. Besides describing floral morphology, number of disc florets per capitulum, number of pollen grains per floret, and P/O ratio were quantified. Furthermore, I defined six floral stages according to anther tube and style morphology to investigate the process of pollen presentation and assessed pollen viability and stigma receptivity. Significant differences were found in the number of disc florets per capitulum (range 19 - 61, average 44.1) and the number of pollen grains in each floret (range 6556 - 11378) among individuals. Secondary pollen presentation was observed as the growing style brushes and pumps pollen grains out of the anther tube. Incomplete protandry was found from the observation of the flowering process and the tests of pollen viability and stigmatic receptivity. High P/O ratio (means \pm SE: 8827 ± 464) was measured which suggested that *B. pilosa* var. *radiata* might be obligately xenogamous.

Key words: Autogamy, *Bidens pilosa* var. *radiata*, Capitulum, Pollen/ovule ratio (P/O ratio), Protandry, Secondary pollen presentation, Stigmatic papillae, Sweeping hairs, Xenogamy

Introduction

Invasion of exotic species is a global phenomenon which often has negative environmental and economic impact on the region being invaded (Mooney and Hobbs, 2000; Pimentel *et al.*, 2000). Therefore, invasion by non-indigenous species has been recognized as one of the most serious threats to global biodiversity (Begon *et al.*, 1996; Vitousek *et al.*, 1997; Shortt and Vamosi, 2012). Reducing population size of invasive species and preventing their spread are critically important in controlling their expansion. Reproduction is necessary for plants to increase population size and to spread into other habitats (Richardson, 2004). Through increasing our understanding of invasive plant reproduction, I will have a better chance to find the most effective way(s) to control them and reduce their environmental threat.

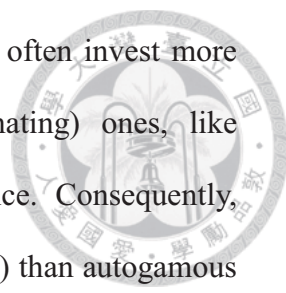
Bidens pilosa L. var. *radiata* Sch. Bip., an annual or perennial herb belonging to Asteraceae, was first collected and documented in 1937 (Sherff, 1937) and recorded in Taiwan in 1984 (Peng *et al.*, 1998; Wu *et al.*, 2004). After being introduced into Taiwan (probably from North America), it is distributed from low to mid elevations throughout the island (Peng *et al.*, 1998) and has become one of the twenty most noxious invasive plants in Taiwan (Chiang *et al.*, 2003). One of the fundamental questions about invasive plants is what makes them invasive (Sutherland, 2004). Reproduction plays an important role in establishing a new colonizing population (Baker, 1955). Plants with efficient reproductive strategies could potentially have a better chance to increase population size and distribution range than those without (Xiao *et al.*, 2011). *B. pilosa* var. *radiata* was found to be able to reproduce vegetatively (Hsu, 2006; Huang, 2008), which might partially contribute to its success in dominating colonized habitats.



In addition to vegetative reproduction, *B. pilosa* var. *radiata* also produces achenes on its capitula by sexual reproduction which could increase genetic diversity. To our knowledge, sexual reproduction and floral biology of *B. pilosa* var. *radiata* in Taiwan have not been studied. A detailed study of the functional morphology of the flowers of this invasive plant would provide clues regarding its mode of sexual reproduction.

Secondary pollen presentation, the relocation of pollen grains from anthers onto another flower organs as pollen presenting organ for pollination, is found in five monocotyledon and 13 dicotyledon families of angiosperms (Howell *et al.*, 1993). It is a typical characteristic of Asteraceae. The apical portion of its style may be externally covered with sterile hairs (called sweeping hairs) which often participate in the pollen presentation process (Ladd, 1994). Three types of secondary pollen presentation have been described in this family, which are correlated with the arrangement of the sweeping hairs (Leins and Erbar, 1990; Erbar and Leins, 1995). Species with hairs only at the tip of the style operated the pump mechanism, those with hairs reaching below the branches of the style performed the brush mechanism, and those with hairs clothing only part of the externals of the style branches conducted an intermediate mechanism. Most species with secondary pollen presentation were also protandrous to avoid self-pollination (Howell *et al.*, 1993; Yeo, 1993; Ladd, 1994; Shivanna, 2003). Therefore, the stylar morphology, pollen viability and stigma receptivity of *B. pilosa* var. *radiata* were studied to better understand the pollen presentation mechanism and to test the hypothesis whether this taxon is protandrous.

Although outcrossing can increase genetic heterogeneity, it also reduces the reproduction assurance. Because their pollen need to be transferred to other



individuals for fertilization, xenogamous (cross-pollinating) plants often invest more resources to male versus female than autogamous (self-pollinating) ones, like producing more pollen grains, to increase reproduction assurance. Consequently, xenogamous species often have higher pollen/ovule ratio (P/O ratio) than autogamous species (Richards, 1997; Shivanna, 2003). Cruden (1977) found significant difference in P/O ratio between xenogamous and autogamous species, and suggested that the P/O ratio of flowering plants could reflect their breeding system (Cruden, 1977; Richards, 1997; Wang and Hu, 2011). Thus, in this study, I also investigated the P/O ratio of the plant to gain a prediction about the possible breeding system of this invasive plant.

This study aims to understand floral biology of *B. pilosa* var. *radiata*, including (1) floret morphology, (2) the process of secondary pollen presentation and (3) the P/O ratio.

Materials and Methods

Plant materials

Plant materials were planted from achenes, which were collected from central Taiwan (23°26' N, 120°36' E, 500 m a.s.l.). Plants were grown in 4 L pots (18.5 cm × 19.5 cm) and placed at an experimental farm of the National Taiwan University (25°00' N, 121°32' E, 15 m a.s.l.). When the plants started flowering, about two to three months after planting, I observed the flowering process and analyzed the adult flowers.

Floral structure

The inflorescence of *B. pilosa* var. *radiata* is a capitulum composed of two types of sessile florets. Five to eight white ray florets (0.5 - 1.5cm long) surround disc florets arising at the same level on a flattened axis, and the whole is surrounded by involucre bracts.

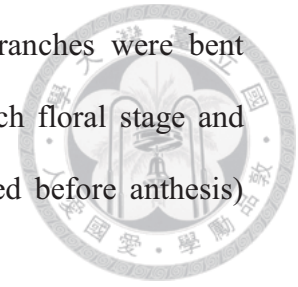
The size of fresh pollen grains was measured with a light microscope equipped with a calibrated ocular micrometer. To examine the detailed morphology of pollen grains, florets were removed from capitula and put in a dry cabinet (SBM-B1B, Bossmen, Taipei, Taiwan) more than 72 hrs, and then pollen grains were spread on metal stubs with double-sided adhesive tape and sputter-coated with gold by a gold-particle coating machine (E101, Hitachi, Tokyo, Japan). The resulting materials were then observed with an SEM (inspect S, FEI, Brno, Czech).

To prevent the dehydration of the styles, a different procedure was used for preparation. Fresh styles were collected, mounted directly on a metal stub with double-sided adhesive tape and observed with a SEM with cryoholder facilitates (TM 1000, Hitachi, Tokyo, Japan) (Tang *et al.*, 2012).

Floral stage and lifespan

Bidens pilosa var. *radiata* is a hermaphrodite and its yellow disc florets are all monoclinous. Six floral stages of a disc floret (A, B, C, D, E, and F) were defined based on the morphology and behavior of anther tube and style (**Figure 3-3**). At stage A, corolla was closed. At stage B, corolla opened, anther tube raised and part of pollen grains were presented at top of anther tube. At stage C, the style elongated and brushed and pumped pollen grains out of anther tube. At stage D, style branches

separated and exposed the stigmatic areas. At stage E, style branches were bent downward. Flower was withered at stage F. The durations of each floral stage and total floral lifespan were monitored daily on a capitulum (marked before anthesis) until the last floret of the capitulum senescence.



Stigma receptivity and pollen viability

At each floral stage, styles were removed from florets and put on a slide for the detection of stigmata receptivity. A droplet of the test solution, mixture of one 15 × 15 mm peroxtesmo Ko peroxidase test paper (Macherey-Nagel, Dueren, Germany) with 1 ml distilled water, was applied onto the stigma (style branches). The stigma was considered receptive if it turned dark (Dafni and Maués, 1998).

At each floral stage, pollen grains were collected from anther tube, spread on a slide, stained by fluorescein diacetate (FDA) solution, and finally examined under a fluorescence microscope (Nikon, Tokyo, Japan). Viable pollen grains would show bright fluorescence when excited by blue illumination (filter set: B-2A excitation filter 450-490 nm and DM 510 dichronic mirror; Nikon, Tokyo, Japan). I counted 200 pollen grains of each sample and determined the pollen viability (= viable pollen number/200). The average of three florets taken from 3 different capitula of the same individual was used to represent the value of that individual, and five individuals were sampled.

Number of pollen grains and pollen/ovule ratio

To estimate the number of pollen grains and P/O ratio, pollen grains of a single

tubular floret were counted with a counter chamber under a microscope. Pollen numbers of two florets from one individual were counted and means were calculated to represent the pollen number of this individual. In total, 20 florets from 10 individuals were counted. The difference of number of pollen grains among 10 individuals was analyzed with ANOVA (general linear model procedure of SAS, release 9.1, SAS Inst. Inc.).

Results

Number of disc florets/capitulum

Among the 58 capitula counted, most (50) capitula had 35 to 55 disc florets, only 5 capitula had disc florets less than 35 and 3 capitula had disc florets more than 55 (**Figure 3-1**). The mean florets number of each capitulum was 44.1 ± 1.0 (means \pm SE, $n = 58$), and there was significant difference in the number of disc florets per capitulum among the ten individuals ($F_{9, 48} = 2.11, p = 0.047$).

Floral morphology

The dissection of a disc floret of *B. pilosa* var. *radiata* is shown in **Figure 3-2A**. A disc floret was composed of five joined petals (forming a tubular corolla), five stamens with coherent brown anthers (forming an anther tube) and separated white filaments (**Figure 3-2 B₁** and **B₂**), a long style with two branches at the apex ascending from the center of the anther tube and an inferior ovary.

Before disc floret anthesis, the anthers dehisce and release pollen grains into the

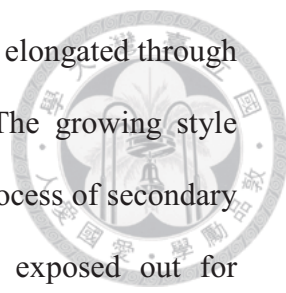
anther tube (**Figure 3-2B₁**). The pollen grains (**Figure 3-2C**), diameter of 32.1 ± 0.5 μm (with spinule) or 27.9 ± 0.8 μm (without spinule), were echinate, globular and tricolporate. After the style grew out of the anther tube, only a few pollen grains would be left in the anther tube (**Figure 3-2B₂**).

Style tip was covered by two kinds of microstructure (**Figure 3-2D**). The tip and abaxial surface of the style branches were covered by longer brushing hairs (or sweeping hairs) (**Figure 3-2D**) while the adaxial surface (the stigmatic area) was occupied with smaller and shorter papillae (**Figure 3-2D**).

Flowering process and floral stage

Flowering of *B. pilosa* var. *radiata* occurred continually in the field. A capitulum of *B. pilosa* var. *radiata* began anthesis with maturation of ray florets. Following up, disc florets opened sequentially, about one whorl per day, from periphery to the centre of a capitulum. The flowering duration from the first disc floret to the final one was approximately four to six days.

According to the flowering process of the floret, six floral stages (A, B, C, D, E, and F) was defined according to the morphology of the floret, and it extended for about 6 days from flower bud to flower withered (**Figure 3-3**). In general, the disc floret started anthesis with the opening of the corolla before 8:00 am. Within 24 hours before anthesis, the corolla was closed and its tip turned yellow. The disc florets with this appearance were defined as at stage A (**Figure 3-3A**). Following stage A, the corolla tube elongated, the tip of the corolla tube separated into five lobes, and the anther tube (with its appendage still closed) grew out of corolla (stage B, **Figure**



3-3B). About 16 hours after stage B (00:00 a.m. on day 2), the style elongated through the anther tube, when was defined as stage C (**Figure 3-3C**). The growing style brushed and pumped the pollen grains out of the anther tube (the process of secondary pollen presentation). Thus, at stage C the pollen grains were exposed out for pollination. After protruding through the anther tube, the style gave off two branches at the apex and then exposed the stigma, this stage was defined as stage D (**Figure 3-3D**). Stage D usually happens around 8:00 am. About 24 hours later (08:00 a.m. on day 4), the style branches bended downward, and the stigma was at the highest position of the floret, the floret was defined as at stage E (**Figure 3-3E**). Finally, at stage F (on day 5 and day 6), anthers and style branches were dehydrated, withered, and shrank back (**Figure 3-3F**).

Stigma receptivity and pollen viability

Results from the peroxidase test revealed that the stigma surface occupied with papillae was the site for the reception of pollen grains and where were not receptive (**Figure 3-4 A and B**) when the style branches were still in the anther tube, i.e. at stage A and B. Surprisingly, although the stigma had not been exposed at stage C, it already appeared receptive (**Figure 3-4C**). The stigma remained receptive after being exposed, at stage D and E (**Figure 3-4 D and E**). Accordingly, the stigma was exposed and receptive for about 2 days.

The change of pollen viability was very different from that of stigma receptivity. Before florets flowering, at stage A, $77.2 \pm 2.1\%$ (means \pm SE, $n = 5$) pollen grains were viable (**Figure 3-5**). After florets open, the percentage of viable pollen decreased gradually from stage B to stage F. At stage F, only $3.5 \pm 1.8\%$ pollen grains were

viable.



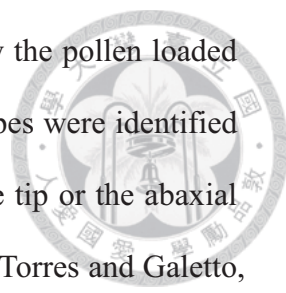
Number of pollen grains and pollen/ovule ratio

Number of pollen grains of a single tubular floret was 8827 ± 464 (means \pm SE, $n = 10$). Significant difference was found in the number of pollen grains in each floret (range 6556-11378) among the 10 individuals ($F_{9, 10} = 6.78, p = 0.003$). Since there is only one ovule in each floret, the average number of the pollen grain per floret can also be used to represent the P/O ratio.

Discussion

Bidens pilosa var. *radiata* has invaded Taiwan and is threatening local biodiversity (Chiang *et al.*, 2003; Wu *et al.*, 2004; Hsu, 2006; Huang, 2008; Wu *et al.*, 2010). The growth and physiology of the plant have been studied (Hsu, 2006; Huang, 2008; Hsu and Kao, 2009) while its sexual reproduction is nearly unknown. According to our observations, the invasive plant produces flowers and sets fruits in field year-round. Thus, sexual reproduction is expected to play an important role in spreading of the plant. This study provides some basic information on floral biology of this invasive plant.

All taxa in the Asteraceae were reported having pollen presenters (Ladd, 1994). Consistent with Ladd's report, secondary pollen presentation was also observed in *B. pilosa* var. *radiata* (**Figure 3-3**). Secondary pollen presentation has been suggested as a mechanism enhancing the efficiency and accuracy of pollen exportation and/or pollen reception (Yeo, 1993). According to which organ is presenting pollen grains,

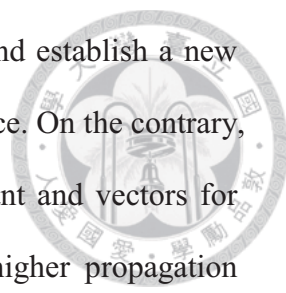


whether pollen is exposed or concealed within a structure, and how the pollen loaded onto the presenters, nine different secondary pollen presentation types were identified in 16 angiosperm families (Howell *et al.*, 1993). In Asteraceae, the tip or the abaxial surface of the style branches are the pollen presenters (Ladd, 1994; Torres and Galetto, 2007). Due to growth of the style, the sweeping hairs located on top of the style or style branches push pollen grains out of anther tube for pollination (Howell *et al.*, 1993; Ladd, 1994), and this process was also found in *B. pilosa* var. *radiata*. According to the location of the sweeping hairs, the presentation mechanisms were divided into pumping, brushing, and a combination of both types (Leins and Erbar, 1990; Yeo, 1993; Leins and Erbar, 2006). In *B. pilosa* var. *radiata*, the tip and the abaxial surface of the style branches were covered by the sweeping hairs (**Figure 3-2D**). Based on Leins and Erbar's (1990, 2006) classification, the pollen presentation mechanism of *B. pilosa* var. *radiata* combined a pump and a brush mechanism which is a typical feature in the Asterales complex (Leins and Erbar, 1990; Yeo, 1993; Leins and Erbar, 2006).

Dichogamous protandry, male matures before female, is common in hermaphroditic floret in the Asteraceae (Faegri and Pijl, 1979; Cerana, 2004). If the flower is protandrous, secondary pollen presentation would promote xenogamy (Howell *et al.*, 1993; Yeo, 1993; Ladd, 1994; Shivanna, 2003). According to our observation of the flowering process of the plant (**Figure 3-3**), the pollen grains were presented out for pollination at stage C (referred as functional male phase). Though the stigma was already receptive at stage C (**Figure 3-4C**), however, it was not exposed until stage D (referred as functional female phase). Because its male phase (stage C) appeared prior to the female phase, the hermaphroditic floret of *B. pilosa* var. *radiata* could be considered as protandrous. However, further assessments revealed

that there was some overlap between functional male and functional female phase. Though pollen viability decreased gradually from stage A to stage F (**Figure 3-5**), there were about 20% of pollen grains which remained viable at stage D during the presence of the functional female phase. Thus, protandry was incomplete in this plant. A similar situation was also found in *Mikania micrantha* (Hong *et al.*, 2008), another invasive weed belonging to the Asteraceae.

Cruden (1977) measured P/O ratio of many taxa with different breeding systems to investigate the relationship between P/O ratio of these plants and their breeding system. It was found that P/O ratio was highest (2108 - 195525, $n = 25$) in obligately xenogamous species and decreased from obligate xenogamy, facultative xenogamy, facultative autogamy, obligate autogamy to cleistogamy. In Asteraceae, the P/O ratio ranged from 262 to 12890 in obligate xenogamy, from 114 to 8214 in facultative xenogamy or facultative autogamy, and from 33 to 373 in obligate autogamy (Erbar and Langlotz, 2005). The P/O ratio of *B. pilosa* var. *radiata* (8827 ± 464) was much higher than that of closely related taxa *B. lemmonii* (113.7) and *B. leptcephala* (998.6 ± 42.6), which were facultative autogamous and facultative xenogamous, respectively (Cruden, 1977; Erbar and Langlotz, 2005). The result suggests that *B. pilosa* var. *radiata* might be obligately xenogamous. In addition, the size (range: 20 - 40 μm) and the morphology of the pollen grains (with many spinules, **Figure 3-2C**) imply that pollen grains of *B. pilosa* var. *radiata* are adapted for adherence to insects vectors (Wodehouse, 1935). It was reported that *B. pilosa* var. *radiata* could not produce achenes in an insect excluded greenhouse (Hsu, 2006). Thus, it is highly possible that *B. pilosa* var. *radiata* is self-sterile or self-incompatible and depends on animals for cross-pollination.



Autogamous (self-pollinating) plants can produce offspring and establish a new population from a single individual, thus have reproductive assurance. On the contrary, xenogamous (cross-pollinating) plants need another individual plant and vectors for pollination. Thus, the autogamous plants are expected to have higher propagation rates than xenogamous ones in closely related taxa. This might be the reason that autogamous taxa were found to be more widespread than xenogamous ones in many closely related taxa (Erbar and Langlotz, 2005). However, xenogamous plants can increase genetic heterogeneity, thus are favored in heterogeneous and variable environments. It was reported that *B. pilosa* var. *radiata* could reproduce asexually by clonal growth (Hsu, 2006; Huang, 2008). Thus, the combination traits of vegetative reproduction and xenogamy might contribute to the widespread distribution of this invasive plant in Taiwan.

In conclusion, *Bidens pilosa* var. *radiata* has the mechanism of secondary pollen presentation. The growing style brushes and pumps the pollen grains out of the anther tube. Results from the observation of the flowering process, the test of stigma receptivity and the measurement of pollen viability revealed that *B. pilosa* var. *radiata* is incomplete protandrous (**Figure 3-6**). The high P/O ratio found in this plant suggested that *B. pilosa* var. *radiata* might be obligately xenogamous.

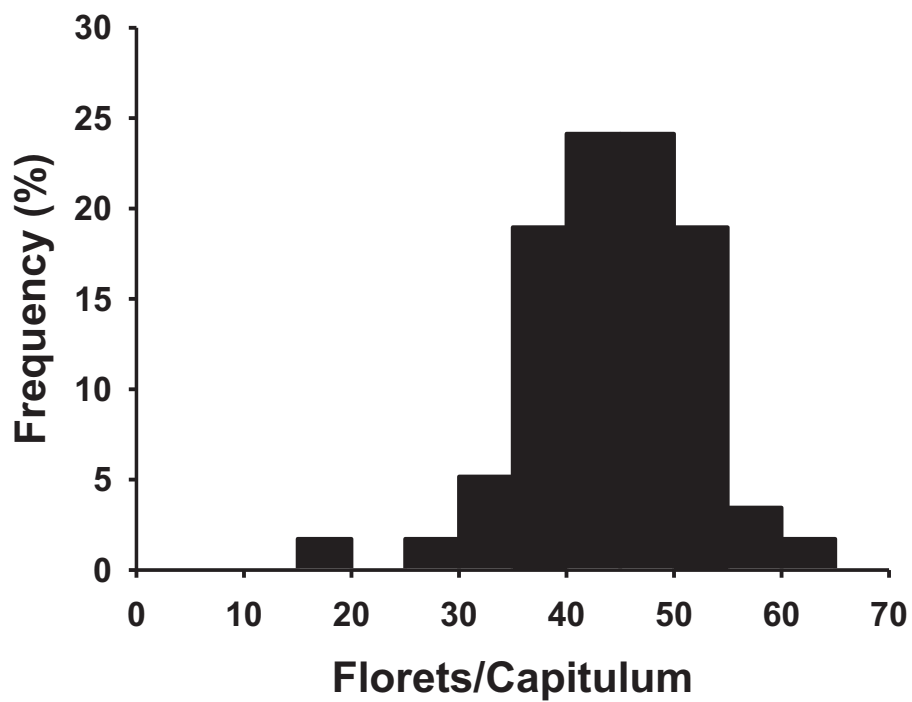


Figure 3-1 Disc florets number in a capitulum of *Bidens pilosa* var. *radiata*. Normal frequency distribution (Kolmogorov-Smirnov test, $D = 0.096$, $n = 58$, $p > 0.15$) of hermaphroditic disc florets number in a capitulum of *Bidens pilosa* var. *radiata*.

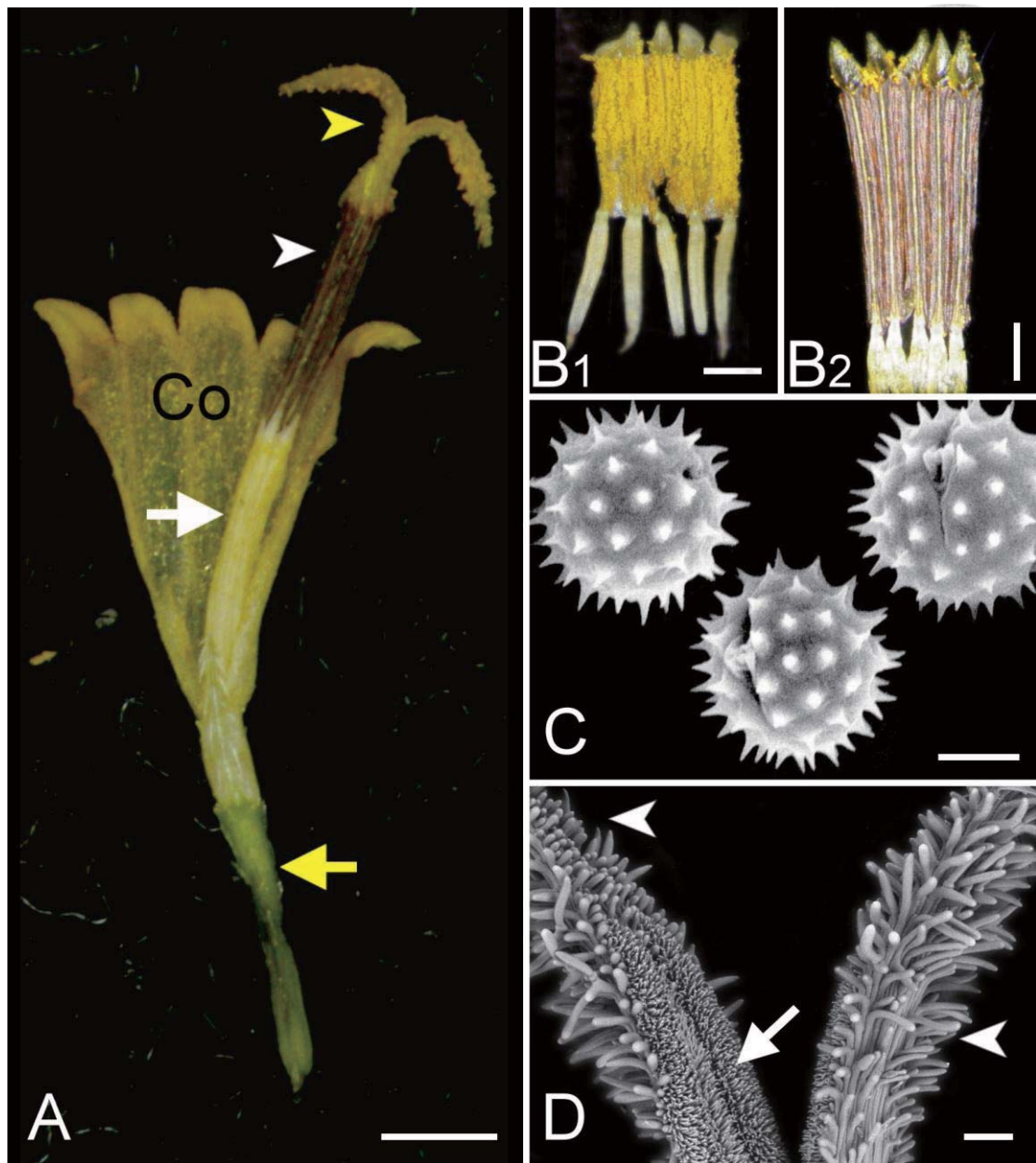


Figure 3-2 Disc floret and its reproductive components of *Bidens pilosa* var. *radiata*. (A) a dissected disc floret consisting five joined petals (forming a tubular corolla, Co), five stamens with united brown anthers (forming an anther tube, white arrowhead) and white filaments free from each other (white arrow), one style with two stylar arms (yellow arrowhead) situated in the center of the anther tube, and an inferior ovary (yellow arrow) (bar = 1 mm); (B₁) a dissected anther tube full of pollen grains before anthesis (bar = 200 μ m); (B₂) a dissected anther tube with few pollen grains remained after the style branches growing out of the anther tube (bar = 200 μ m);

(C) the equatorial view (the right two) and the polar view (the left one) of the tricolporate pollen grains with echinate ornaments (bar = 10 μm); (D) the branches of style tip with sweeping hairs (or brushing hairs) on the tip and abaxial surface (white arrowhead) and stigmatic papillae on the adaxial surface (white arrow) (bar = 100 μm).



Figure 3-3 The Six floral stages of *Bidens pilosa* var. *radiata* during anthesis. (A) Stage A: corolla still closed (arrowhead); (B) Stage B: corolla opened, the anther tube (arrowhead) raised above the corolla, pollen grains presented at the top of the anther tube (due to pollen pumping); (C) Stage C: style elongated, stylar branches (arrowhead) begin to separate and brush pollen grains out of the anther tube; (D) Stage D: stylar branches separated and exposing the stigmatic areas (arrowhead); (E) Stage E: the style branches are bent downward; (F) Stage F: flower withered. All figures share the same scale bar in F (bar = 1 mm).

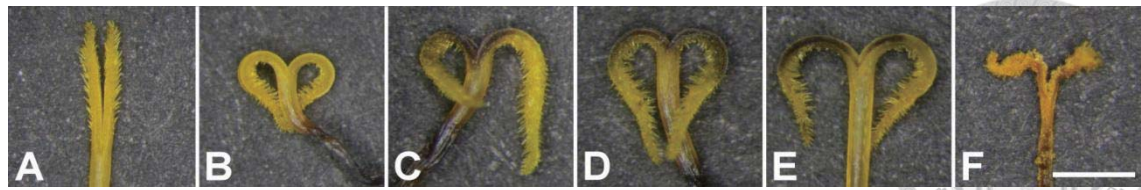


Figure 3-4 Stigma receptivity of *Bidens pilosa* var. *radiata* at six floral stages. The peroxidase test of stigma receptivity of florets at different floral stages (A - F). The stigma was receptive, appeared dark, at stages C, D, E, but not at stages A, B, F. All figures share the same scale bar as in F (bar = 1 mm).

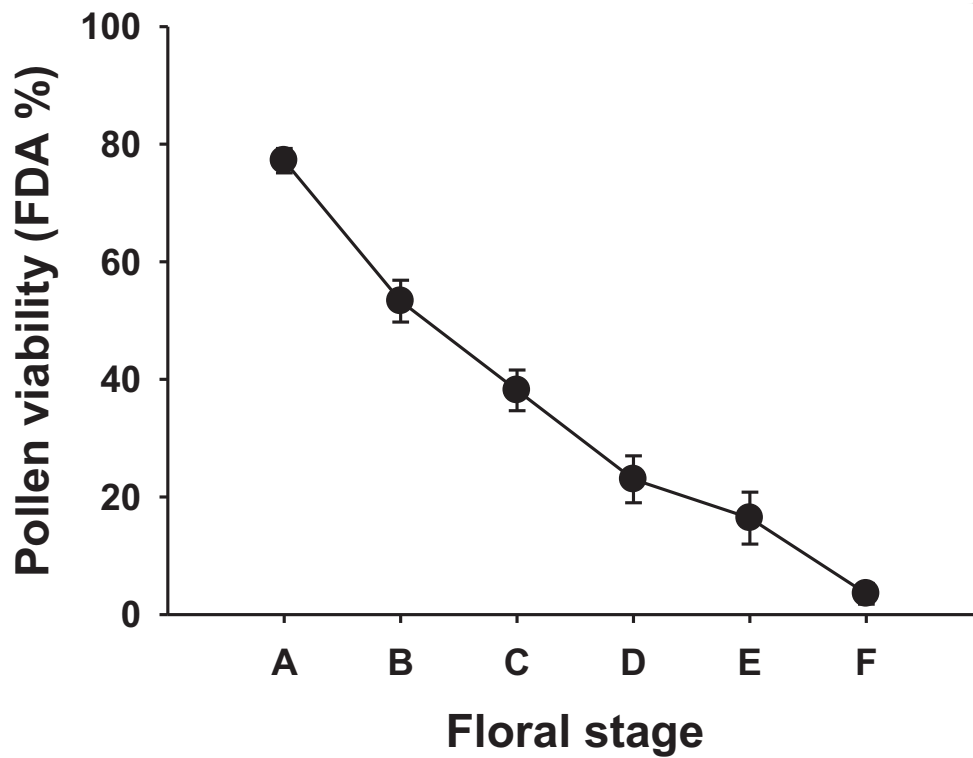


Figure 3-5 Pollen viability of *Bidens pilosa* var. *radiata* at six floral stages. Mean pollen viability (means \pm SE, $n = 5$) of *Bidens pilosa* var. *radiata* at six floral stages (as defined in Figure 3-3).



Floral stage	A	B	C	D	E	F
Start time	Day 0 8:00	Day 1 8:00	Day 2 0:00	Day 3 8:00	Day 4 8:00	Day 5 to Day 6
Floret morphology						
Stigma receptivity						
Pollen viability (FDA %)	77.2 ± 2.1	53.3 ± 3.6	38.1 ± 3.5	23 ± 4.0	16.4 ± 4.4	3.5 ± 1.8

Figure 3-6 Summaries of six floral stages. Time, floral morphology (bar = 1 mm), stigma receptivity (bar = 1 mm) and pollen viability of a tubular floret of *Bidens pilosa* var. *radiata* at six floral stages. This figure combining the result of Figure 3-3 - Figure 3-5 and the description about the time of six floral stages in the text.



Chapter 4

Breeding systems of the three varieties of *Bidens pilosa*, var. *minor*, var. *pilosa* and var. *radiata*

(This chapter has been published in *Weed research* 54: 162-168, 2014)

摘要

先前研究發現當三種咸豐草變種種植在溫室中時，僅大花咸豐草無法結實，小白花鬼針與白花鬼針則可結實，因此本研究進行套袋實驗，以了解三個變種是否具不同的有性繁育系統。

本研究比較三個變種的結實量、花粉-雌蕊交互作用、花粉/胚珠比，結果發現小白花鬼針與白花鬼針的頭狀花序在套袋後有 45 - 55% 的結實率，但大花咸豐草的頭狀花序在套袋後幾乎沒有成熟瘦果產生，顯示大花咸豐草自交結實率低。接著觀察花粉-雌蕊交互作用，結果發現在套袋處理下，大花咸豐草僅 1.5% 的雌蕊有花粉管成功延伸入花柱，而小白花鬼針與白花鬼針則各有 76% 與 52% 的雌蕊有花粉管成功延伸。由此可知：大花咸豐草具有強烈的自交不親和以防止自交。再者，大花咸豐草、小白花鬼針與白花鬼針三者的花粉/胚珠比分別為：8189、2053 與 1613。這些結果均支持此三個變種有不同繁育系統的假設：入侵植物大花咸豐草為高度自交不親和，而小白花鬼針與白花鬼針則自交親和。

關鍵詞：咸豐草、繁育系統、入侵植物、花粉/胚珠比、花粉-雌蕊交互作用、自交不親和

Abstract

In previous studies, it was observed that var. *radiata* was not able to set achenes in a greenhouse but var. *minor* and var. *pilosa* could set achenes. Therefore, I conducted bagging treatment in this study to understand whether the three varieties had different breeding systems.

Differences in achene set in bagged versus open-pollinated capitula, pollen-pistil interactions and the pollen/ovule (P/O) ratio were investigated among these three varieties. In contrast with the resulting 45–55% achene set in each bagged capitulum of var. *minor* and var. *pilosa*, almost no mature achenes (1–2 achenes) were found in bagged capitula of var. *radiata*, showing that var. *radiata* has a low capability of autonomous achene production. In bagged capitula, only 1.5% of pistils of var. *radiata*, but 76% and 52% of those of var. *minor* and *pilosa*, respectively, had pollen tubes penetrating into their styles, suggesting that var. *radiata* has strong self-incompatibility mechanisms preventing autonomous autogamy. The P/O ratio, with mean values of 8189, 2053, and 1613 for var. *radiata*, var. *minor* and var. *pilosa* respectively, were obtained. These results support the hypothesis that the three varieties bear different breeding systems; the var. *radiata* is highly self-incompatible, whereas the var. *minor* and var. *pilosa* are self-compatible.

Keywords: Hairy beggar-ticks, Mating system, Invasive plant, Pollen/ovule ratio (P/O ratio), Pollen-pistil interaction, Self-incompatibility

Introduction

Identifying factors that contribute to the success of introduced species has been an important subject of invasion biology (Goulson and Rotheray, 2012). Being able to reproduce outside the native range is essential for the establishment of alien species (Richardson *et al.*, 2000a; Richardson, 2004; Sutherland, 2004; van Kleunen *et al.*, 2010), thus, the study of reproduction is fundamental for understanding biological invasion. Many studies on the reproductive biology of invasive plants have recognised that breeding systems play a crucial role in contributing to the successful invasion of alien species into non-native ranges (Petanidou *et al.*, 2012; Ward *et al.*, 2012; Munguia-Rosas *et al.*, 2013).

Bidens pilosa L. (hairy beggar-ticks) is a common invasive plant in tropical and subtropical regions (Cui and He, 2009). There are three varieties of *B. pilosa* in Taiwan: *B. pilosa* var. *minor* (abbreviated as var. *minor*), *B. pilosa* var. *pilosa* (abbreviated as var. *pilosa*) and *B. pilosa* var. *radiata* (abbreviated as var. *radiata*) (Peng *et al.*, 1998). *B. pilosa* var. *radiata*, first recorded in Taiwan in 1984 (Peng *et al.*, 1998; Wu *et al.*, 2004), is an aggressive weed throughout the lowland of the island and is listed as one of the top 20 most serious invasive plants in Taiwan (Chiang *et al.*, 2003). It is probably native to the United States, but is now a common weed in North and South America, North Africa and south Asia. The var. *pilosa* and var. *minor* were also introduced and share many morphological traits and a similar habitat with var. *radiata* in Taiwan. These two varieties are cosmopolitan and widely distributed throughout tropical and subtropical regions (Ballard, 1986). Thirty years ago, var. *minor* widely distributed in Taiwan. However, after var. *radiata* being introduced into Taiwan, var. *radiata* has become a serious invasive plant and dominant over the other two varieties within 30 years. This leads one to question what makes *B. pilosa* var.

radiata more dominant than the other two varieties in Taiwan now. Limited information is available to answer this question.

Hsu (2006) reported that var. *radiata* produced flowers but had no achene set when grown in an insect-excluded glasshouse. In a glasshouse experiment, I found that var. *minor* and var. *pilosa* produced achenes, but in contrast, var. *radiata* produced almost no achenes (Y.-L. Huang, pers. comm.), this suggested that the three varieties might have different breeding systems. To our knowledge, sexual reproduction of the three varieties has not been compared. Therefore, the aim of this study was to investigate aspects associated with sexual reproduction of the three varieties. It is known that all three varieties are hermaphrodite (Peng *et al.*, 1998) and produce achenes in the field. I thus propose that the var. *radiata* is self-incompatible and depends on vectors for pollination, whereas var. *minor* and var. *pilosa* are self-compatible and are capable of autonomous achene production.

Two mechanisms preventing selfing are often found in species of the Asteraceae (Hiscock, 2000; Shivanna, 2003; Hong *et al.*, 2007; Hong *et al.*, 2008; Allen *et al.*, 2011). Dichogamous protandry, in which anthers dehisce before the stigma becomes receptive, is considered as a mechanism for reducing the impact of pollen-pistil interference on pollen import and export (Barrett, 2002) and thus might reduce rates of self-fertilisation and enhance outcrossing (Harder *et al.*, 2000). Huang *et al.* (2012) reported that var. *radiata* has incomplete protandry. Self-incompatibility (SI), in which self-fertilisation and inbreeding are prevented by the gene products of the S-locus that prevent reproduction between individuals sharing one or more SI alleles (de Nettancourt, 2001), is considered another evolutionary strategy to avoid self-fertilisation and inbreeding (Richards, 1997; de Nettancourt, 2001). In the Asteraceae, the self-incompatibility response usually occurs at the stigmatic surface;

either self-pollen grains do not germinate or the growth of pollen tubes is arrested (Allen *et al.*, 2011). To further interpret results of bagging treatments, I also investigated whether the three varieties have different degrees of protandry and/or SI.

This study had three objectives concerning the sexual reproductive biology of three varieties of *B. pilosa*. Firstly, pollination treatments were conducted to detect self-fertility. Secondly, the behaviour of pollen and stigma was investigated to explore the existence of protandry and self-incompatibility, and finally, I aimed to quantify P/O ratios.

Materials and Methods

Plant materials

Bidens pilosa (Asteraceae) is an annual or perennial herb with erect stems and opposite leaves. Three varieties of *B. pilosa*, var. *minor*, var. *pilosa* and var. *radiata*, distributed on roadsides and cultivated fields from low to middle elevations have been reported in Taiwan (Peng *et al.*, 1998). The morphological differences among the three varieties can only be distinguished by their ray florets. Ray florets are absent in the var. *pilosa*, whereas the var. *radiata* and var. *minor* both have five to eight ray florets on each capitulum. The ray florets of var. *radiata* are usually longer than 10 mm, but those of var. *minor* are shorter than 8 mm.

Achenes of the three varieties collected from several populations in central Taiwan (23°26' N, 120°36' E, 500 m a.s.l.) where they were found growing together were germinated, planted (10 individuals for each variety) in 4 L pots (18.5 cm × 19.5 cm) and placed in the experimental farm of the National Taiwan University (25°00'N, 121°32'E, altitude 15 m a.s.l.). Experiments were performed when plants started to

flower, 2-3 months after planting.

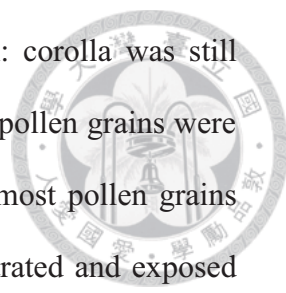


Pollination treatments

Pre-anthesis capitula of the same individual were assigned for bagging and open pollination (control) treatments. For each variety, ca. 30 capitula (referred to as bagged capitula) at the bud stage were enclosed in fine nylon-mesh netting bags (7 × 9 cm) for the bagging treatment, whilst another 30 capitula (referred to as open-pollinated capitula) were marked and left untreated as the control treatment. To prevent the loss of mature achenes, I also enclosed open-pollinated capitula in nettings after all florets withered. After 3 - 4 weeks of treatments, I harvested the fruiting capitula. During the experimental period, some capitula were mildewed or damaged, consequently in total, 28 capitula of var. *radiata*, 30 of var. *minor*, and 27 of var. *pilosa* from the bagging treatment and 30 capitula of each variety from the control treatment were harvested. Following harvesting, the numbers of undeveloped ovules, mature and immature achenes were counted. Because there was only one ovule in each floret, the sum of mature achenes, immature achenes and undeveloped ovules represent the floret number per capitulum. The percentage of mature achene set (% achene set) per capitulum in bagged and open pollination treatments was calculated as the number of mature achenes divided by the number of florets. The percentage achene set in bagged capitula was used to evaluate the capability of autonomous achene production in each variety.

Stigma receptivity and pollen viability

Six floral stages from flower bud to withered flowers were defined according to



anther tube and style morphology (Huang *et al.*, 2012). Stage A: corolla was still closed. Stage B: corolla was opened, anther tube raised and part of pollen grains were presented at top of anther tube. Stage C: the style elongated and most pollen grains were presented at top of anther tube. Stage D: style branches separated and exposed the stigmatic areas. Stage E: style branches were bent downwards. Stage F: flower was withered. Stigma receptivity and pollen viability of each floral stage were investigated. A droplet of the test solution, a mixture of one 15 × 15 mm peroxtesmo Ko peroxidase test paper (Macherey-Nagel, Dueren, Germany) with 1 mL of distilled water, was applied onto the stigma for the detection of receptivity. The stigma was considered receptive if the stigma turned dark (Dafni and Maués, 1998). To test pollen viability, pollen grains were collected from the anther tube, spread onto a slide, stained by fluorescein diacetate (FDA) solution and examined under a fluorescence microscope (Nikon, Tokyo, Japan). Viable pollen grains showed bright fluorescence when excited by blue illumination (filter set: B-2A excitation filter 450 - 490 nm and DM 510 dichronic mirror; Nikon). I counted 200 pollen grains for each floret and determined the pollen viability (viable pollen number/200). The mean for three florets taken from three different capitula of the same individual was used to represent the value of that individual, and five individuals for each variety were sampled.

Pollen-pistil interaction

One to three capitula (at the bud stage) of a plant were assigned to the bagging treatment ($n = 10$ plants) and another set of capitula marked as open pollination treatment (or control, $n = 10$ plants). When the majority of stigmas on each capitulum were exposed for more than 24 h, pistils were collected and fixed in Carnoy's solution (95 % ethanol: chloroform: acetic acid = 6:3:1) for 24 h, and cleared in 8 N NaOH for

more than 24 h. Pistils were then washed with distilled water, placed on a slide and stained with 0.1% aniline blue in 0.1 M K_3PO_4 for 10 min. Samples were mounted with glycerine solution (glycerine:distilled water, 1:1 v/v) and examined under a fluorescence microscope (Nikon) with excitation by UV illumination (11000v2 excitation filter 340 - 380 nm, emission 420 nm).

Twenty pistils with pollen grains deposited on their stigma from each individual were observed for the determination of the degree of self-incompatibility. The status of these pistils could be classified into three types. Type I pistils had only non-germinated pollen grains on the stigma (**Figure 4-1A**). Type II pistils had germinated pollen grains but whose pollen tubes did not penetrate into the styles due to callose formation (**Figure 4-1B**). Type II pistils might also have non-germinated pollen grains on its surface. Type III pistils had pollen tubes growing straight and penetrating the style (**Figure 4-1C**). Type III pistils might also have non-germinated pollen grains on the stigma and/or germinated pollen grains but whose pollen tubes did not penetrate the style. The percentage of each type of pistil for bagged and open-pollinated capitula was calculated.

Pollen/ovule ratio

The mean number of pollen grains from two tubular florets sampled from each plant ($n = 10$ for each variety) was calculated to represent the pollen number per floret for each plant. The number of pollen grains was estimated using a counter chamber under a microscope. Because each floret has only one ovule, the pollen number of each floret was used to represent the P/O ratio.

Statistical analysis

The hermaphrodite disc florets ($n = 27 - 30$) and percentage achene set ($n = 27 - 30$) between bagging and open pollination treatments or among varieties of the same treatments were analysed using analysis of variance (ANOVA; general linear model procedure of SAS, release 9.1, SAS Inst. Inc.). The percentage achene set was arcsine-transformed prior to ANOVA to fit the assumption for parametric tests. The pollen/ovule ratio (P/O ratio; $n = 10$), pollen viability ($n = 5$) among three varieties and the percentage of the three types of pistils ($n = 10$) between bagging and open pollination treatments and among varieties of the same treatments were analysed with the Kruskal-Wallis Test. If the null hypothesis was rejected after the analysis of ANOVA or Kruskal-Wallis Test, the Student-Newman-Keuls test was used for multiple comparison tests.

Results

Achene set from bagging and control treatments

Among the three varieties, var. *radiata* had significantly fewer hermaphrodite disc florets per capitulum than the other two varieties (**Table 4-1**). Only two of the 28 bagged capitula of var. *radiata* produced achenes and within these two capitula, only one and two mature achenes were found, respectively, and these achenes failed to germinate. In contrast, all of the bagged capitula of var. *minor* ($n = 30$) and var. *pilosa* ($n = 27$) produced about 30 achenes per capitulum (**Table 4-1**). Thus, a significant difference was found among the three varieties in the percentage achene set of bagged capitula, with var. *minor* having the highest (55.5%), var. *radiata* the lowest (0.3%) and var. *pilosa* an intermediate value (45.8%) (**Table 4-1**). In contrast, no significant

difference was found among the three varieties in the percentage achene set in the open-pollinated capitula. The achene set of open-pollinated capitula of var. *radiata* was 57.7%, that of var. *minor* was 62.6% and that of var. *pilosa* was 45.8%. Significant differences in percentage achene set between bagged and open-pollinated capitula were both found in var. *radiata* and var. *pilosa*, but not found in var. *minor* (**Table 4-1**).

Stigma receptivity and pollen viability

On pistils of all three varieties, the stigmas were receptive from stage C to E. Although the stigma was already receptive at stage C, it was not exposed until stage D (referred to as the functional female phase; **Figure 4-2**). For all three varieties, pollen grains were presented for pollination at stage C (referred to as the functional male phase). However, the pollen viabilities of all three varieties were not highest at stage C. The pollen viabilities were about 69.8% - 77.2% at stage A. In the following, pollen viabilities of all three varieties gradually decreased from stage A to F (**Figure 4-2**). When compared at the same stage, no significant difference was found in pollen viabilities among the three varieties.

Pollen-pistil interaction

Comparing the florets on open-pollinated capitula, a similar pattern of pollen-pistil interaction was found in all three varieties, with most florets having type III pistils (72.5% - 78.5%) and almost no florets having type II pistils (var. *radiata*: 1.5%; var. *minor* and var. *pilosa*: 0%) (**Table 4-2**). A similar pattern was found in bagged capitula of var. *minor*, 76.0% pistils of var. *minor* were type III, 24% pistils

were type I and no type II pistil was found. However, the pattern varied in bagged capitula of var. *radiata* and var. *pilosa*. In bagged capitula of var. *radiata*, most florets were of type I (66.0%), least of type III (1.5%) and intermediate of type II pistils (32.5%). Florets of bagged capitula of var. *pilosa* showed a similar percentage of type I (48%) and type III (52.0%) pistils, and no type II pistil was found (**Table 4-2**).

Pollen/ovule ratio

The pollen/ovule ratio (P/O ratio) of var. *radiata* was about 8189, and there was a significant difference in the P/O ratio among these 10 plants ($\chi^2 = 18.5$, $P = 0.03$). The P/O ratio of var. *minor* and var. *pilosa* were 2053 and 1613, respectively. Different from var. *radiata*, no difference was found in the P/O ratio among ten individuals of var. *minor* and var. *pilosa* (**Table 4-3**). Among the three varieties, var. *radiata* had a significantly higher P/O ratio than the other two varieties ($\chi^2 = 20.7$, $P < 0.001$), whereas the P/O ratio of var. *minor* and that of var. *pilosa* were not significantly different (**Table 4-3**).

Discussion

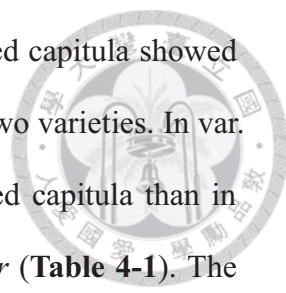
Sexual reproduction may play an important role in determining plant invasiveness (Daehler, 1998; Rambuda and Johnson, 2004; van Kleunen and Johnson, 2007; van Kleunen *et al.*, 2008; Harmon-Threatt *et al.*, 2009; Petanidou *et al.*, 2012; Ward *et al.*, 2012). In this study, I found that three varieties of *B. pilosa* with different invasiveness in Taiwan now had significant differences in autonomous achene production, the pattern of pollen-pistil interaction in capitula following bagging treatment and the P/O ratio, associated with sexual reproduction. These results support

the hypothesis that the three varieties have different breeding systems.

Achene production in capitula following bagging treatments (**Table 4-1**) revealed that var. *minor* and var. *pilosa* are capable of self-pollination, whereas var. *radiata* has a low capability of autonomous achene production. Without the aid of pollinators, var. *radiata* is unlikely to produce achenes. This explains the contrasting results of achene production between var. *radiata* and the other two varieties when grown sympatrically in an insect-excluded glasshouse (previous observation). The size and morphology of the pollen grains of *B. pilosa* var. *radiata* imply that pollen grains are adapted for adhesion to insect vectors (Huang *et al.*, 2012). It is presumed that the dependence on insect pollination might partially contribute to the evolution of showy ray florets in var. *radiata*. In fact, the showy ray florets of this variety were the reason for its introduction into Taiwan, to increase honey production by bees.

Dichogamy was a mechanism preventing plants from selfing (Shivanna, 2003). Protandry, the anthers dehisce before the stigma becomes receptive, is often found in species of Asteraceae (Bertin and Newman, 1993; Barrett, 2002). Incomplete protandry was found in all three varieties with pollen viability decreasing from stage A to F, indicating a similar degree of protandry. However, the incomplete protandry did not prevent autonomous achene production in var. *minor* and var. *pilosa*. Accordingly, protandry is not the main mechanism preventing *B. pilosa* var. *radiata* from autonomous achene reproduction. The result of pollen-pistil interaction explains why there was no achene production (**Table 4-1**) and provides evidence that self-incompatibility was the mechanism preventing autonomous achene production in bagged capitula of var. *radiata* (Richards, 1997; de Nettancourt, 2001; Allen *et al.*, 2011).

Although var. *minor* and var. *pilosa* had autonomous achene set in bagged

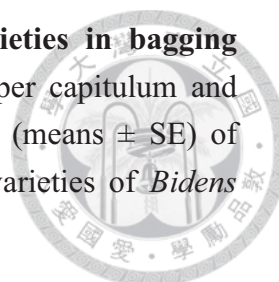


capitula, comparison of the percentage achene set in open-pollinated capitula showed that the efficiency of autonomous achene set differed between the two varieties. In var. *pilosa*, the percentage achene set was significantly lower in bagged capitula than in open-pollinated capitula, but no difference was found in var. *minor* (**Table 4-1**). The pattern of pollen-pistil interactions found in these two varieties is consistent with this result (**Table 4-2**). Significantly, more type I but fewer type III pistils were observed in bagged capitula than in open-pollinated capitula of var. *pilosa*, whereas, in var. *minor*, bagged capitula and open pollination florets had a similar percentage of type I and type III pistils. It is suggested that in var. *pilosa*, the lower percentage achene set in bagged than in open-pollinated capitula was caused by an increasing failure of pollen germination on the stigmas of bagged florets. Accordingly, var. *pilosa* might have a higher level of self-incompatibility than var. *minor* (Allen *et al.*, 2011).

Within an evolutionary lineage, plants producing more pollen are more likely to set more outcrossed seeds (Cruden, 1977; Mione and Anderson, 1992). In these three varieties of *B. pilosa*, the results of P/O ratio correspond with their breeding systems, high P/O ratio and high SI for var. *radiata* while low P/O and low self-incompatibility for var. *minor* and var. *pilosa*.

In conclusion, the three varieties of *B. pilosa* in Taiwan have different breeding systems. The var. *radiata* was self-sterile, while var. *minor* and var. *pilosa* were self-fertile. Self-incompatibility was the mechanism preventing var. *radiata* from producing achenes autogamously.

Table 4-1 Disc floret number and achene set of three varieties in bagging experiment. Number of hermaphrodite disc florets per capitulum and the percentage of mature achene set (% achene set) (means \pm SE) of bagged or open-pollinated (open) capitula in three varieties of *Bidens pilosa*.



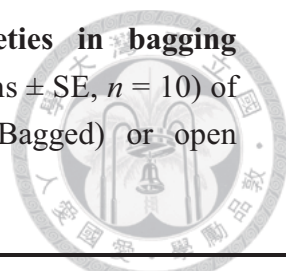
Variety	Number of hermaphrodite disc florets		% achene set [†]	
	Bagged	Open	Bagged	Open
var. <i>radiata</i>	41.36 \pm 1.68 ^b	46.57 \pm 0.92 ^{b*}	0.3 \pm 0.2 ^c	57.7 \pm 2.8*
var. <i>minor</i>	55.70 \pm 1.75 ^a	58.23 \pm 1.32 ^a	55.5 \pm 2.9 ^a	62.6 \pm 2.2
var. <i>pilosa</i>	57.78 \pm 2.50 ^a	53.93 \pm 2.19 ^a	45.8 \pm 3.1 ^b	61.9 \pm 2.9*

Means within each column followed by different letters differed significantly ($P < 0.05$).

*: significant difference ($P < 0.05$) between bagged and open pollination treatments of the same variety.

[†] : % achene set was calculated as the number of mature achenes divided by the number of florets.

Table 4-2 Percentage of three pistil type of three varieties in bagging experiment. Percentage of three types of pistils (means \pm SE, $n = 10$) of three *Bidens pilosa* varieties receiving bagging (Bagged) or open pollination (Open) treatments.



Variety	Type I		Type II		Type III	
	Bagged	Open	Bagged	Open	Bagged	Open
var. <i>radiata</i>	66.0 \pm 9.1 ^a	26.0 \pm 2.7*	32.5 \pm 8.8 ^a	1.5 \pm 0.8 ^{a*}	1.5 \pm 0.8 ^c	72.5 \pm 2.5*
var. <i>minor</i>	24.0 \pm 7.0 ^b	21.5 \pm 6.2	0 \pm 0 ^b	0 \pm 0 ^b	76.0 \pm 7.0 ^a	78.5 \pm 6.2
var. <i>pilosa</i>	48.0 \pm 7.1 ^a	26.5 \pm 7.0*	0 \pm 0 ^b	0 \pm 0 ^b	52.0 \pm 7.1 ^b	73.5 \pm 7.0*

Type I pistils: with only non-germinated pollen grains on the stigma.

Type II pistils: having germinated pollen grains but the pollen tube not penetrating stigma due to callose formation.

Type III pistils: with pollen tubes growing straight and penetrating the stigma.

Means within each column followed by different letters differed significantly ($P < 0.05$).

*: significant differences ($P < 0.05$) between bagging and open pollination treatments of the same variety.

Table 4-3 Pollen/ovule ratio of three varieties. Pollen/ovule ratio ($n = 10$) of each hermaphrodite disc floret in the three varieties of *Bidens pilosa*.

Variety	Pollen/Ovule ratio		χ^2	P
	Means \pm SE	Range		
var. <i>radiata</i>	8189 \pm 523 ^a	5833 - 11472	18.15	0.03
var. <i>minor</i>	2053 \pm 162 ^b	1200 - 2622	5.92	0.75
var. <i>pilosa</i>	1613 \pm 152 ^b	956 - 2378	11.41	0.25

Means followed by different letters differed significantly ($P < 0.05$).

χ^2 and P values: significant differences ($P < 0.05$) of pollen number among 10 individuals (the data of each individual was mean of pollen/ovule ratio of two disc florets).

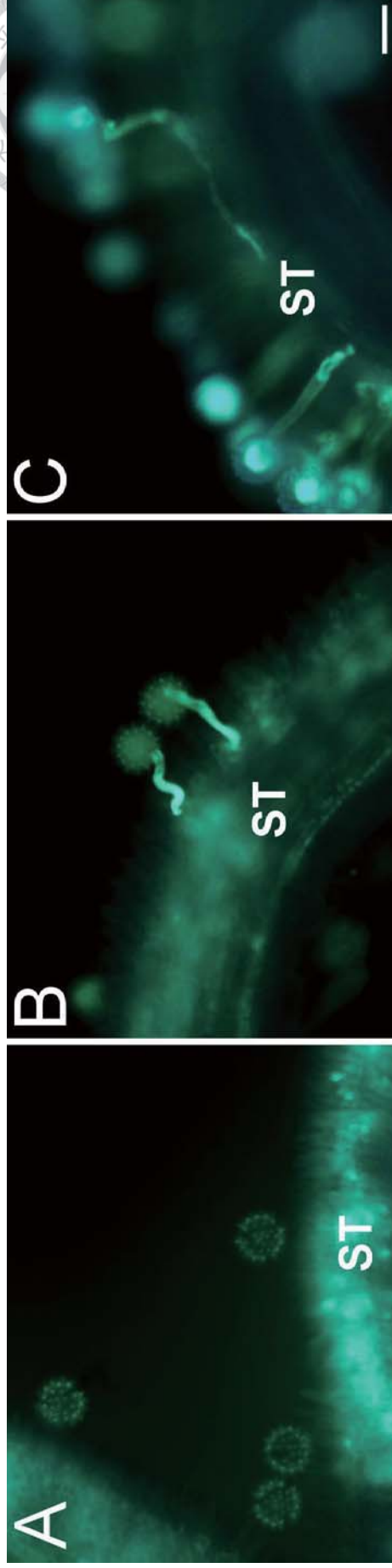


Figure 4-1 Fluorescent pictures of three types of pistils. Fluorescent pictures representing three types of pistils found in pollen-pistil interaction in florets of *Bidens pilosa*. (A) Type I pistil, with only non-germinated pollen grains on the stigma (ST), (B) Type II pistil, with germinated pollen grains but due to callose formation, the pollen tube not penetrating the stigma, and (C) Type III pistil, with pollen tubes growing straight and penetrating the stigma (bar = 50 μm).

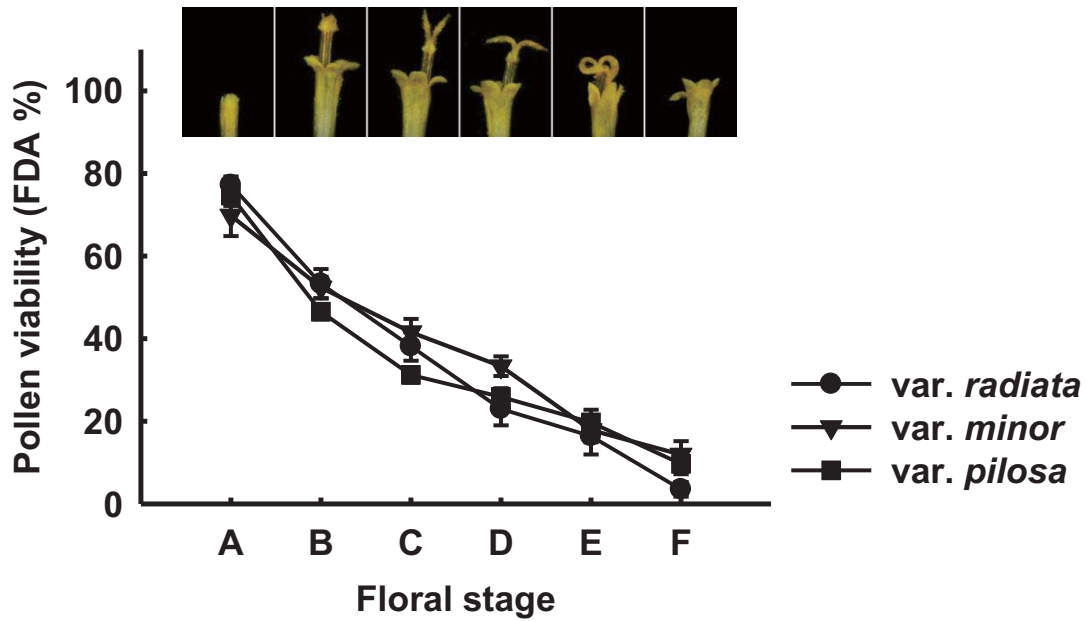


Figure 4-2 Pollen viability of three varieties at six floral stages. Changes in the mean percentage of pollen viability (means \pm SE, $n = 5$) and the status of the pistil (pictures) of florets at six different floral stages in three varieties of *Bidens pilosa*: corolla closed (stage A); corolla open, anther tube raised and part of pollen grains presented at top of anther tube (stage B); the style elongated and most pollen grains presented at top of anther tube (stage C); style branches separated and the stigmatic areas exposed (stage D); style branches bent downward (stage E); flower withered (stage F).





Chapter 5

Life history traits of the three varieties of *Bidens pilosa*, var. *minor*, var. *pilosa* and var. *radiata*

摘要

植物是否能成功地入侵某一地區，其生活史特徵扮演一個很重要的角色。為了釐清大花咸豐草和另外兩變種的生活史特徵是否有差異，本研究比較三種咸豐草的開花時間、瘦果產量、瘦果重、發芽率、植株壽命，以期找出大花咸豐草優於另外二變種，並成為臺灣嚴重入侵植物的原因。

結果顯示：小白花鬼針與白花鬼針較大花咸豐草早開花，且實驗初期的瘦果產量較大花咸豐草高。然而，種植十個月後，所有的小白花鬼針與白花鬼針植株均死亡，但大花咸豐草仍有五株存活，並有分株(ramet)，且繼續開花結瘦果，種植一年後大花咸豐草所累積的瘦果產量便超過另外兩者。因此，小白花鬼針與白花鬼針應為一年生的物種，且其側枝無法形成分株。部分大花咸豐草植株壽命可超過一年，且存活個體的側枝能形成分株，證實先前的推測（第二章）：大花咸豐草可以用枝條行無性繁殖。雖然，小白花鬼針與白花鬼針所結瘦果較大花咸豐草的瘦果重、較快發芽，但三者最終的發芽率則無顯著差異。

由此推測：大花咸豐草的植株壽命長、形成分株的能力強，產生較多的瘦果是造成大花咸豐草比另外兩變種在臺灣佔優勢的重要特徵，也可能是使大花咸豐草變成入侵植物的重要生物特徵。

關鍵字：開花時間、營養繁殖、生活史特徵、瘦果發芽、瘦果產量

Abstract

Characteristics of life history were considered important traits related with plant invasiveness. In order to clarify traits that make *B. pilosa* var. *radiata* more dominant than the other two varieties, var. *minor* and var. *pilosa*, in Taiwan, I planted the three varieties and compared their life history traits (blooming time, achene production, achene weight, germination rate and life span).

I found that var. *minor* and var. *pilosa* flowered earlier and produced more achenes than var. *radiata* in 300 days after germination. However, after planted for more than ten months, all individuals of var. *minor* and var. *pilosa* died while five individuals of var. *radiata* survived and grew many ramets, which flowered and set achenes afterwards. Consequently, var. *radiata* accumulated significantly more achenes than the other two varieties after planted for more than one year. The results revealed that var. *minor* and var. *pilosa* are annual while some of individuals of var. *radiata* are perennial and are able to grow ramets. The ability of growing ramets indicated that var. *radiata* has clonal growth potential. *B. pilosa* var. *minor* and var. *pilosa* had heavier achenes which germinated faster than achenes of var. *radiata*. However, no significant difference was found among the three varieties in final germination percentage of achenes.

The results suggested that longer life span and being able to grow ramets might be important traits making var. *radiata* more dominant than var. *minor* and var. *pilosa* in Taiwan.

Keywords: Blooming time, Clonal growth, Life history traits, Achene germination, Achene production

Introduction

Invasive species often cause harmful impacts to biodiversity of some ecosystem (Begon *et al.*, 1996), and affect regional economics and society, thus, the invasive biology has become an important subject not only in ecological study but also in economy (Pimentel *et al.*, 2000). Successful invaders often have some traits promoting their invasiveness (van Kleunen *et al.*, 2010). Identifying these traits might help us finding ways to control and manage the invasive species and identifying potential invaders. Accordingly, effective action could be taken to reduce their negative effect.

Bidens pilosa L. (hairy beggar-ticks) is a common invasive plant in tropical and subtropical regions (Cui and He, 2009). Three varieties of *B. pilosa* were found in Taiwan: *B. pilosa* var. *minor* (abbreviated as var. *minor*), *B. pilosa* var. *pilosa* (abbreviated as var. *pilosa*) and *B. pilosa* var. *radiata* (abbreviated as var. *radiata*) (Peng *et al.*, 1998). *B. pilosa* var. *radiata* is probably native to the United States, but is now a common weed in North and South America, North Africa and south Asia. The first record of var. *radiata* in Taiwan is in 1984 (Peng *et al.*, 1998; Wu *et al.*, 2004). After that, it has become rampant in the past three decades. Being an aggressive weed throughout the lowland, var. *radiata* is listed as one of the top 20 most serious invasive plants in Taiwan (Chiang *et al.*, 2003). *B. pilosa* var. *minor* and var. *pilosa* were also introduced and share many morphological traits and similar habitats with var. *radiata* in Taiwan. These two varieties are cosmopolitan and widely distributed throughout tropical and subtropical regions (Ballard, 1986). Thirty years ago, var. *minor* widely distributed in Taiwan. However, after var. *radiata* being introduced into Taiwan, var. *radiata* has become a serious invasive plant and dominant over var. *minor* and var. *pilosa* in Taiwan lowland. This leads one to question what makes *B.*

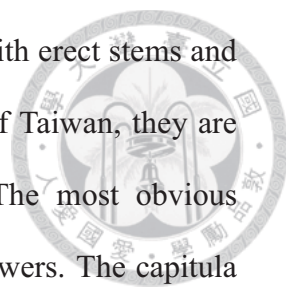
pilosa var. *radiata* more dominant than the other two varieties in Taiwan lowland now. Limited information is available to answer this question.

Characteristics of life history were considered important traits related with plant invasiveness (Rejmánek and Richardson, 1996; Ozinga *et al.*, 2007). Successful invaders often have a distinct suite of life-history traits (Rejmánek and Richardson, 1996; Hamilton *et al.*, 2005). For examples, a positive correlation between early flowering and local plant abundance has been found (Lloret *et al.*, 2005). Plants producing more achenes were considered a better colonizer (Richardson, 2004). Achene mass represent the chance of successful dispersal by a species into an establishment opportunity and gives an indication of a seedling's ability to survive a range of hazards (Westoby, 1998). A study comparing life-history traits of 29 pine species demonstrated that plants with high relative growth rate of seedling, producing small achenes and having short generation time invaded disturbed habitats easier (Grotkopp *et al.*, 2002). Sutherland (2004) investigated ten life-history traits of weeds and found that life span was the most significant trait that differed in invasive and non-invasive plants.

The objective of this study was to compare the life history traits of the three varieties of *B. pilosa*. Specifically, I compared their blooming time, total achene production, achene weight, achene germination rate and life span. I hypothesize that the invasive var. *radiata* flowers earlier, produces more and lighter achene with higher germination rate and has longer life span than var. *minor* and var. *pilosa*.

Materials and Methods

Plant materials



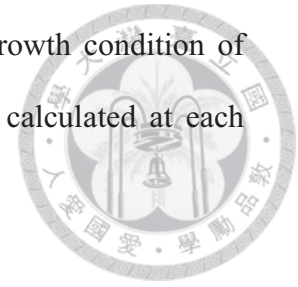
Bidens pilosa L. (Asteraceae) is an annual or perennial herb with erect stems and opposite leaves. Three varieties of *B. pilosa* were listed in Flora of Taiwan, they are var. *minor*, var. *pilosa*, and var. *radiata* (Peng *et al.*, 1998). The most obvious differences in morphology among these three varieties are their flowers. The capitula of var. *minor* and var. *radiata* are radiate (with ray florets), but the capitula of var. *pilosa* are discoid (without ray florets). All these three varieties have 19 - 61 yellow disc florets, which are actinomorphic, hermaphroditic and incompletely protandrous (Huang and Kao, 2014). Only var. *minor* and var. *radiata* have 5 - 8 sterile ray florets, which are zygomorphic and without androecium. However, the size of their ray florets is different. The ray florets of var. *radiata* are often longer than 10 mm, while the ray florets of var. *minor* are often shorter than 8 mm (Peng *et al.*, 1998).

Achenes of these three varieties were collected from central Taiwan (23°26' N, 120°36' E, 500 m a.s.l.) where the three varieties grow sympatrically. Achenes were put on moisturized filter paper in petri dish for germination, and then ten seedlings with similar size were transplanted into 4L (18.5 cm×19.5 cm) pots (1 seedling pot⁻¹) and placed in National Taiwan University on 23rd January, 2013. Because plant growth was space limited after October, 2013, I transported the life plants out from pots and planted them into experimental farm of National Taiwan University.

Blooming and achene production

Blooming date of each individual was recorded. And the mean days from achene germinating to plant blooming of 10 individuals for each variety were calculated as flowering time. Then, after plants started to set achene, capitulum and achene of each individual were collected once a week until the plant died. Achene number of each

capitulum was counted and these achenes were weighted. The growth condition of plants was examined and then survival rate of each variety was calculated at each collection.



Achene germination

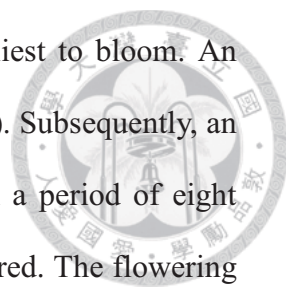
I used the achenes collected from field of central Taiwan to conduct the germination experiment. Achenes were put on moisturized filter paper in a petri dish (25 achenes/petri dish; six petri dishes/variety) which were put in incubator with day/night: 10/14 hrs and 30 °C /25 °C . I checked the achene and recorded the germination rate every day until total achenes germinated or no more achene would germinate.

Statistical analysis

The differences of flowering time, capitulum and achene production, achene weight and achene germination rate of each date among three varieties were analyzed using analysis of variance (ANOVA) (general linear model procedure of SAS, release 9.1, SAS Inst. Inc.). The achene germination rate of each date was arcsine-transformed prior to ANOVA to fit the assumption for parametric tests. If the null hypothesis was rejected after the analysis of ANOVA ($P < 0.05$), the Student-Newman-Keuls test was used for multiple comparison tests.

Results

Flowering time



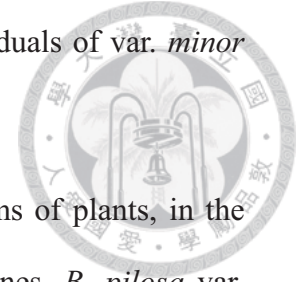
Among the three varieties, *B. pilosa* var. *pilosa* was the earliest to bloom. An individual of var. *pilosa* started to flower on 25th March (**Table 5-1**). Subsequently, an individual of var. *minor* started to flower three days later. Within a period of eight days, the ten individuals of var. *pilosa* and var. *minor* all had flowered. The flowering behavior of var. *radiata* was quite different from that of the other two varieties. *B. pilosa* var. *radiata* started to flower on 5th April, later than var. *minor* and much later than var. *pilosa*. Significant difference in mean days for plants to flower after germination was found between var. *radiata* and the other two varieties ($F_{2, 27} = 35.29$, $P < 0.0001$, **Table 5-1**). In addition, it took a period of 25 days for all the individuals ($n = 10$) of var. *radiata* to flower (**Table 5-1**).

Capituloum and achene production

Achenes matured about three weeks after plants started to flower. During the 52 weeks of collection period, var. *radiata* produced 330 ± 103 capitula (means \pm SE) per individual with 7254 ± 2232 achenes, which are significantly more than var. *minor* (2039 ± 259) and var. *pilosa* (1714 ± 138) (**Table 5-2**; capitulum: $F_{2, 27} = 4.6$, $P = 0.02$; achenes: $F_{2, 27} = 5.7$, $P < 0.01$).

In addition to their differences in the total achene production, the achene production patterns of these three varieties were also quite different. According to the survival rate of these three varieties, I divided the 52 weeks of collection into three periods: Period I was from 23rd April to 4th June, 2013 (the first seven weeks, all ten individuals of three varieties alive in this period), Period II was from 5th June to 29th October, 2013 (the next 21 weeks, the 8th - 28th week, when some individuals of three varieties dead in this period), and Period III was from 30th October, 2013 to 15th April,

2014 (the last 24 weeks, the 29th - 52nd week, when all ten individuals of var. *minor* and var. *pilosa* dead in this period).



Flowers were first seen on the apical meristem on main stems of plants, in the following, more and more shoots started to flower and set achenes. *B. pilosa* var. *minor* generated 43 ± 4 capitula with 1230 ± 59 achenes in Period I and an obvious peak of capitulum and achene production was found on 28th May and 4th June (**Figure 5-1 A and D**). In Period II, var. *minor* generated many capitula (85.3 ± 25.8) (**Figure 5-1A**), but the achene production was much less than that in Period I (809.2 ± 257.1) (**Figure 5-1D**). All individuals of var. *minor* died in Period III, therefore, no capitula and achenes of var. *minor* were generated in Period III (**Figure 5-1 A and D**). Similar pattern was found in var. *pilosa*. There was also an obvious peak of capitulum and achene production found on 28th May and 4th June (**Figure 5-1 B and E**) in Period I. Totally, var. *pilosa* generated 50 ± 4 capitula with 1355 ± 88 achenes in Period I. In Period II, var. *pilosa* generated less capitula (31 ± 11) and achenes (359 ± 123) than that in Period I (**Figure 5-1 B and E**). In the following, all individuals of var. *pilosa* died in Period III, no capitula and achenes of var. *pilosa* were generated in Period III (**Figure 5-1 B and E**). The capitulum and achene production patterns of var. *radiata* were quite different from those of var. *minor* and var. *pilosa*. *B. pilosa* var. *radiata* generated only 15 ± 2 capitula with 345 ± 53 achenes in Period I and no obvious peak of capitulum and achene production were found in this period (**Figure 5-1 C and F**). There were 26 ± 9 capitula and 387 ± 140 achenes generated in late Period II (**Figure 5-1 C and F**). In Period III, var. *radiata* generated 289 ± 102 capitula with 6522 ± 2238 achenes (**Figure 5-1 C and F**). An obvious peak of capitulum and achene production of var. *radiata* was found in this period from 28th January to 11th March, 2014.

B. pilosa var. *minor* and var. *pilosa* accumulated capitulum and achenes quickly in the beginning (capitulum: **Figure 5-2A**, achene: **Figure 5-2B**), it was found that most achenes of var. *minor* and var. *pilosa* were produced in Period I (**Figure 5-2B**). In contrast, var. *radiata* accumulated much less capitula and achenes than the others in Period I, the most capitula and achenes of var. *radiata* were produced in Period III because of the ramets formation (**Figure 5-2**).

After growing two to three pairs of leaves, the axillary shoots of var. *minor* and var. *pilosa* started to flower and set achenes. In Period II, most of capitula and achenes of var. *minor* and var. *pilosa* were collected from axillary shoots. During Period II var. *minor* generated many capitula (**Figure 5-1A**), but few mature achenes were found (**Figure 5-1D**). It was found that the achene/capitulum of var. *minor* was less in Period II than in Period I (**Table 5-3**). Similar pattern was found in var. *pilosa* (**Figure 5-1 B and E and Table 5-3**). In contrast to of var. *minor* and var. *pilosa*, most of axillary shoots of var. *radiata* did not flower until late Period II, but grew continuously to increase their nodes and leaves and became ramets. As a result, the number of accumulated capitulum and achene almost did not increase in the beginning of Period II (**Figure 5-2**). Until late September, 2013, the ramets of var. *radiata* started to flower and set achenes again.

In Period III, all individuals of var. *minor* and var. *pilosa* were dead (**Figure 5-3**), thus, no capitula and achenes of these two varieties were collected in this period. The ramets of var. *radiata* formed in Period II started to flower and set achenes in Period III. Accordingly, most of capitula and achenes of var. *radiata* were produced in Period III (**Figure 5-1 C and F**). Therefore, finally var. *radiata* accumulated significant more capitula and achenes than the other two varieties in the Period III (**Figure 5-2**).

Achene weight

Because ten individuals of each variety were all alive in Period I, I weighted and compared the achenes collected in Period I (14th May to 28th May, 2013). The achene weight of var. *radiata* was 1.07 ± 0.05 g 1000⁻¹ achenes which was significantly less than that of var. *minor* and var. *pilosa*, 1.65 ± 0.04 g 1000⁻¹ achenes and 1.57 ± 0.02 g 1000⁻¹ achenes, respectively ($F_{2,27} = 4.6$, $P = 0.0001$).

Survival rate

Some plants started to die after 4th June (**Figure 5-3**). All ten individuals of var. *minor* and var. *pilosa* gradually died in Period II. In the following, all ten individuals of var. *pilosa* died in late Period II, and all ten individuals of var. *minor* died at the end of Period II. Some individuals of var. *radiata* also died in Period II, and only five individuals were still alive in Period III. And these life individuals lived longer than one year.

Germination rate

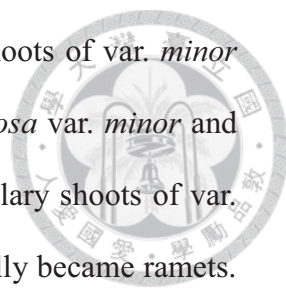
The cumulated achene germination percentage of var. *radiata* was significant less than that of var. *minor* and var. *pilosa* from day 2 to day 7 during the 10 days of germination period (**Figure 5-4**). Although achenes of var. *radiata* germinated later, almost all achenes of these three varieties germinated in nine days after the treatment. Consequently, no significant difference was found in the final germination percentage of achenes of the three varieties ($F_{2,15} = 1.15$, $P = 0.34$).

Discussion

Differences in life history traits were found among the three varieties of *B. pilosa* which might contribute to their differences in invasiveness in Taiwan. A positive correlation between early flowering and local plant abundance has been found (Lloret *et al.*, 2005). In general, invasive plants have early reproductive age (Rejmánek and Richardson, 1996). However, in this study I found that the flowering time (days after germination) of invasive var. *radiata* was significantly later than non-invasive var. *minor* and var. *pilosa* (**Table 5-1**). Plants with different life forms also have different flowering phenology. According to the result of this study, var. *radiata* is perennial but var. *minor* and var. *pilosa* are annual. Therefore, the difference of flowering time between var. *radiata* and var. *minor* and var. *pilosa* might be because they have different life forms.

Due to the fact that var. *minor* and var. *pilosa* flowered earlier than var. *radiata*, var. *minor* and var. *pilosa* accumulated more achenes than var. *radiata* in the early period flowering (Period I and II). However, results from this study demonstrated that total achene production of var. *radiata* was significantly higher than var. *minor* and var. *pilosa* after these plants were cultivated for more than one year. Generating more achene might potentially confer the species ability to increase population size locally and to disperse widely. Therefore, the number of offsprings produced by an individual was found positively correlated with its invasiveness (van Kleunen *et al.*, 2010). Our results are consistent with this correlation.

Higher achene production in var. *radiata* than in the other two varieties was mainly caused by ramet formation ability and perennial life span of var. *radiata*. Although all these three varieties grew axillary shoots, the growth pattern of axillary shoots of var. *radiata* differs from that of var. *minor* and var. *pilosa*. After growing



two to three nodes with two to three pair of leaves, the axillary shoots of var. *minor* and var. *pilosa* started to flower and set achenes. Therefore, *B. pilosa* var. *minor* and var. *pilosa* flowered continuously in Period II. In contrast, the axillary shoots of var. *radiata* continuously growing nodes and producing leaves and finally became ramets. One individual of var. *radiata* could form more than 20 ramets. Subsequently, these ramets flowered and set achenes and contributed to large increment of achene production of var. *radiata* in Period III.

Compared to Period I, significant reduction in achene production was found in Period II in all three varieties. However, the reason causing the reduction differs between var. *radiata* and the other two varieties. Most of shoots of var. *minor* and var. *pilosa* started to dry out after flowering, and after the dried out of shoots, the individual died. Less survival individuals of var. *minor* and var. *pilosa* thus contributed to the leading reason for few achene set in Period II. In addition, few mature achenes on each capitulum were also found in var. *minor* and var. *pilosa* in Period II. In combination, these two factors were mainly responsible for few achenes set by var. *minor* and var. *pilosa* in Period II. However, in var. *radiata*, axillary shoots continued vegetative growth without producing flower buds until late Period II. Therefore, almost no achenes of var. *radiata* were collected until late Period II.

It was found that invasive var. *radiata* had lighter achenes than the other two varieties. Small achene mass is correlated with increased achene output (relative to producing large achenes from a fixed finite resource) (Henery and Westoby, 2001), and is significantly and uniquely correlated with invasion success (Hamilton *et al.*, 2005). Non-native plants having small achenes are likely to be successful invaders in new regions where habitats have been disturbed by human activities (Rejmánek and Richardson, 1996; Grotkopp *et al.*, 2002; Lloret *et al.*, 2004), and the abundant

production of small and long-lived achenes that are well dispersed is a characteristic of plants that are adapted for rapid colonization of disturbed habitat (Grove, 1992). The main habitats of these three varieties were in disturbed localities (Peng *et al.*, 1998). Thus smaller achenes would favor var. *radiata* in disturbed habitat.

The ability to produce many ramets not only enable var. *radiata* to produce more achenes in Period III, but also give var. *radiata* potentials of vegetative propagation and clonal growth. Clonal growth is vegetative production of numerous ramets that remain physically connected by stolon or rhizome internodes for a variable period of time. Clonal species can therefore develop into large interconnected structures consisting of an undetermined number of ramets that can quickly expand horizontally and colonize the surrounding area efficiently. Therefore, clonal growth was a common trait for many aggressive invasive plants (Liu *et al.*, 2006). Comparing to var. *minor* and var. *pilosa*, var. *radiata* was found having high local dominance and persistence in roadsides, disturbed habitats and cultivated fields and being an aggressive weed in lowland throughout the island after introduced into Taiwan. The clonal growth ability might be the most important reason make var. *radiata* become dominant over var. *minor* and var. *pilosa* and become an invasive plant in Taiwan now.

In addition, vegetative propagation and clonal growth ability also provide reproductive assurance. In general, self-compatible species were considered as good colonizers because they could sexually produce offsprings without the presence of other neighbors (Baker, 1955). However, the selective advantage of self-fertilization may be reduced when other mechanisms provide reproductive assurance (Vallejo-Marin and O'Brien, 2007). In my previous study comparing the breeding system of these three varieties, it was found that var. *radiata* is self-incompatible while var. *minor* and var. *pilosa* are self-compatible (Huang and Kao, 2014).

Therefore, the clonal growth ability provides the self-incompatible var. *radiata* reproductive assurance.

B. pilosa var. *radiata* did not flower earlier and the germination rates of its achenes were not higher than the other two varieties. These two characteristic did not consist with my hypothesis. However, the perennial life form and the ability of vegetative propagation are two of the important traits making var. *radiata* displacing many populations of var. *minor* and var. *pilosa* in Taiwan now.

Table 5-1 Blooming of three varieties. Juvenile period (days from achene germinating to first flower blooming of a plant) and blooming date (the first flower blooming date of plants) (means \pm SE, $n = 10$) of three varieties of *Bidens pilosa*.

Varieties	Juvenile period (days)	Blooming date
var. <i>minor</i>	67.6 \pm 0.5 ^b	28 th March - 3 rd April
var. <i>pilosa</i>	64.6 \pm 0.7 ^b	25 th March - 1 st April
var. <i>radiata</i>	80.7 \pm 2.3 ^a	5 th April - 29 th April

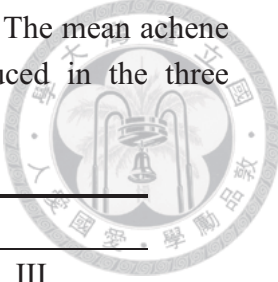
Means followed by different letters differed significantly ($P < 0.05$).

Table 5-2 Capitulum and achene production of three varieties. The total number of capitulum and achene (means \pm SE (range), $n = 10$) produced by an individual of three varieties of *Bidens pilosa* from 23rd April, 2013 to 15th April, 2014.

Varieties	Number of capitulum per individual	Number of achene per individual
var. <i>minor</i>	128 \pm 26 ^b (34 -224)	2039 \pm 259 ^b (1139 - 3190)
var. <i>pilosa</i>	80 \pm 12 ^b (39 -149)	1714 \pm 138 ^b (1109 - 2533)
var. <i>radiata</i>	330 \pm 103 ^a (11 -917)	7254 \pm 2232 ^a (286 - 18184)

Means followed by different letters differed significantly ($P < 0.05$).

Table 5-3 Achene/capitulum in three periods of three varieties. The mean achene number on each capitulum (means \pm SE, (*n*)) produced in the three periods of collection of three varieties of *Bidens pilosa*.



Variety	Period		
	I	II	III
var. <i>minor</i>	34.0 \pm 1.2 (10)	9.9 \pm 0.7 (6)	N.A.
var. <i>pilosa</i>	29.6 \pm 0.5 (10)	10.4 \pm 1.7 (8)	N.A.
var. <i>radiata</i>	23.5 \pm 1.6 (10)	13.3 \pm 1.3 (8)	24.9 \pm 1.9 (5)

Period I was from 23rd April to 4th June, 2013 (the 1st - 7th week).

Period II was from 5th June to 29th October, 2013 (the 8th - 28th week).

Period III was from 30th October, 2013 to 15th April, 2014 (the 29th - 52nd week).

N.A.: All individuals of var. *minor* and var. *pilosa* were dead, and no capitulum was collected in Period III. Thus, achene/capitulum was not available.

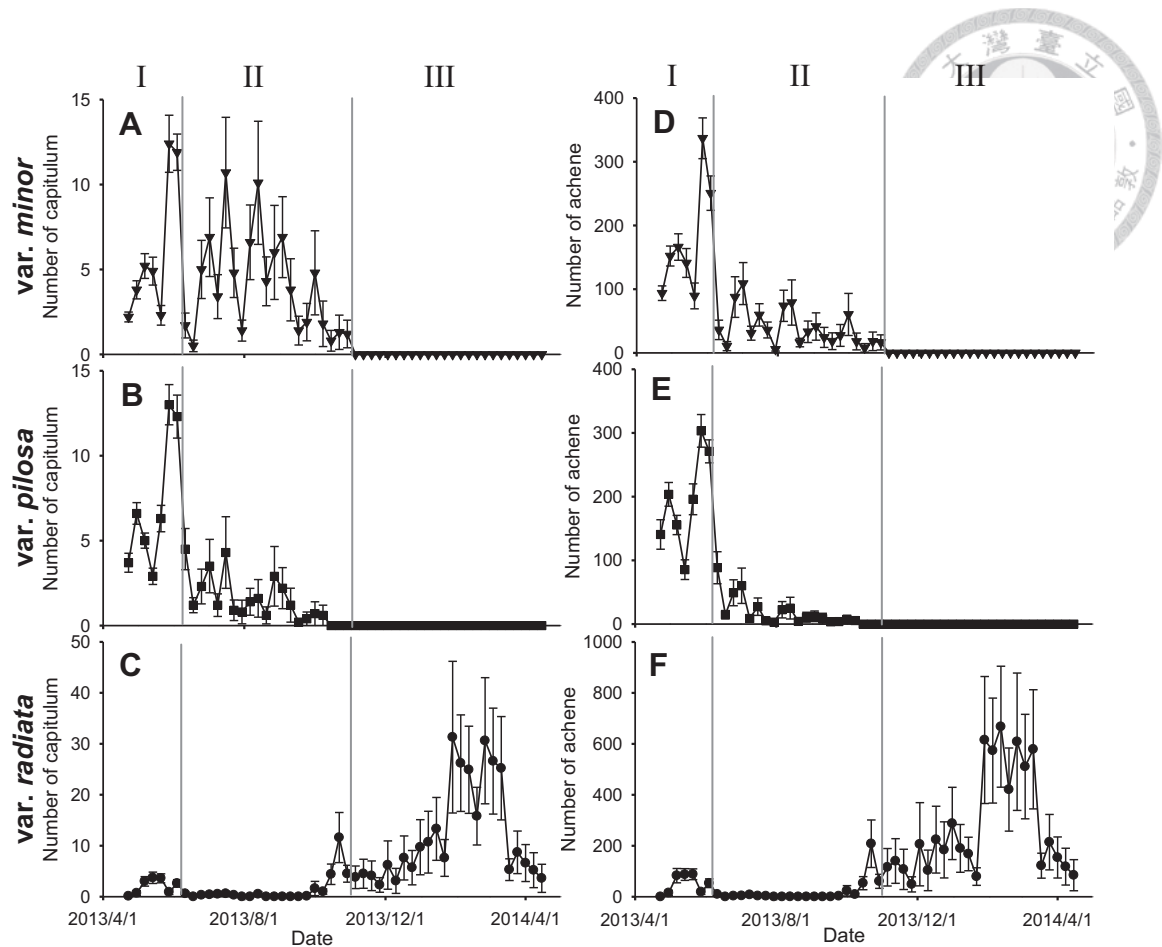


Figure 5-1 **Capitulum and achene production of three varieties.** Capitulum (ABC) and achene (DEF) production (means \pm SE, $n = 10$) of cultivated plants of three varieties of *Bidens pilosa* in each week from 23rd April, 2013 to 15th April, 2014. The vertical gray lines separate the total collecting period into three periods (I, II, and III). Period I was from 23rd April to 4th June, 2013 (the 1st - 7th week), Period II was from 5th June to 29th October, 2013 (the 8th - 28th week), and Period III was from 30th October, 2013 to 15th April, 2014 (the 29th - 52nd week).

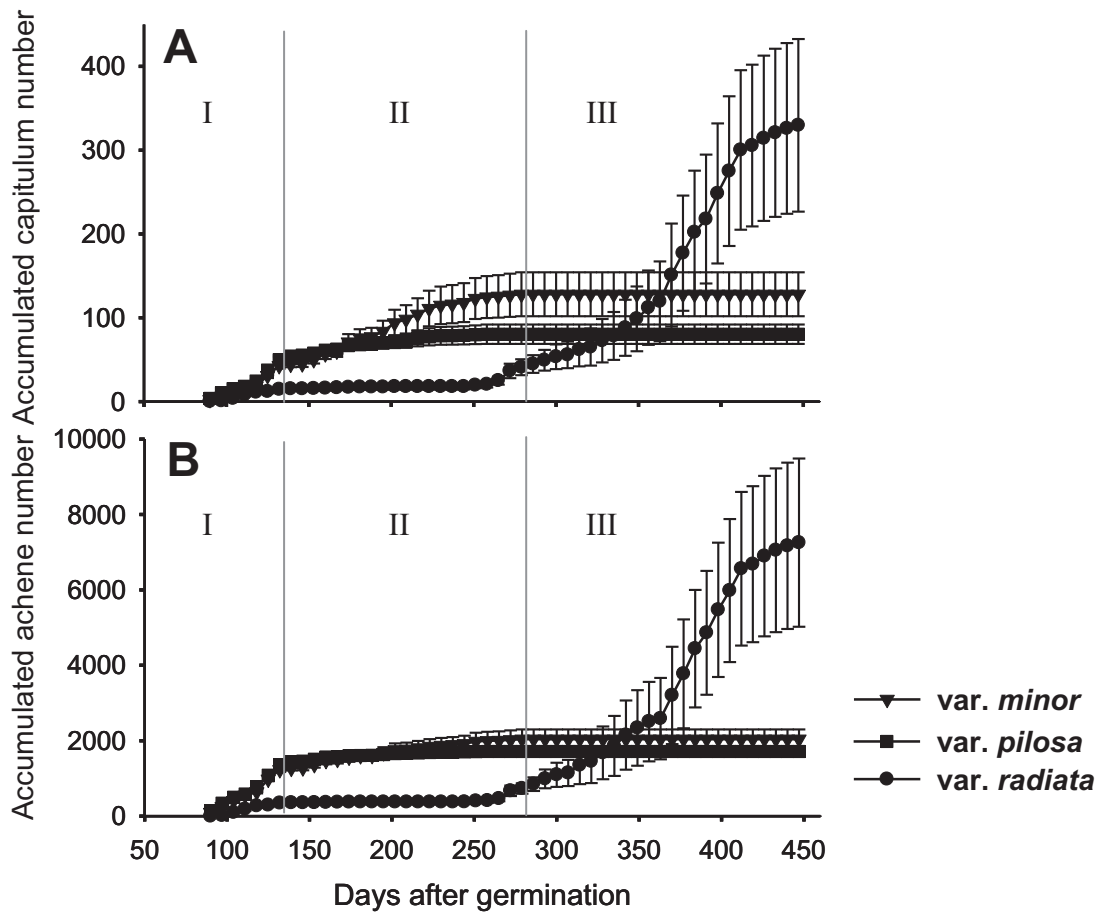


Figure 5-2 Accumulated capitulum and achene production of three varieties. Accumulated capitulum (A) and achene (B) production (means \pm SE, $n = 10$) of cultivated plants of three varieties of *Bidens pilosa*. The vertical gray lines separate the total collecting period into three periods (I, II, and III). Period I was from 23rd April to 4th June, 2013 (the 1st - 7th week), Period II was from 5th June to 29th October, 2013 (the 8th - 28th week), and Period III was from 30th October, 2013 to 15th April, 2014 (the 29th - 52nd week).

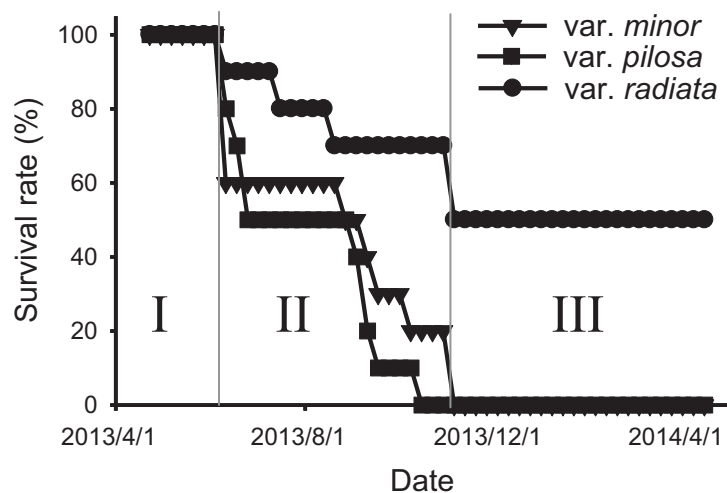


Figure 5-3 Survival rates of three varieties. The survival rates of ten cultured individuals of three varieties of *Bidens pilosa* planted from 23rd January, 2013 to 15th April, 2014. The vertical gray lines separate the total collecting period into three periods (I, II, and III). Period I was from 23rd April to 4th June, 2013 (the 1st - 7th week), Period II was from 5th June to 29th October, 2013 (the 8th - 28th week), and Period III was from 30th October, 2013 to 15th April, 2014 (the 29th - 52nd week).

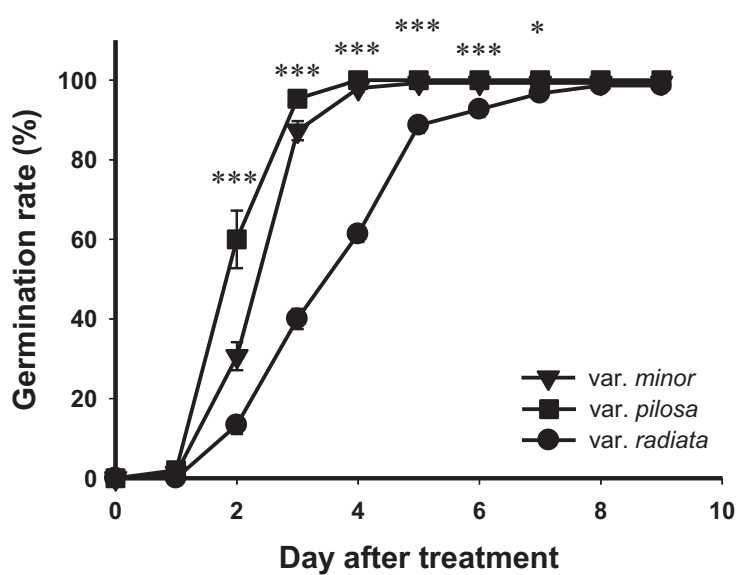


Figure 5-4 Germination rates of three varieties. Germination rates (means \pm SE, $n = 6$) of achenes of three varieties of *Bidens pilosa* (Significant difference on each day: *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).



Chapter 6

**Chromosomal number of populations of the
three varieties of *Bidens pilosa*, var. *minor*,
var. *pilosa* and var. *radiata***

摘要

先前的研究發現大花咸豐草不僅在舌狀花的有無，和舌狀花的形態上與另外兩變種有所不同，三變種的繁育系統亦不相同。此外，許多性狀在大花咸豐草個體間的變異大，而這些性狀在小白花鬼針或白花鬼針的個體間則無顯著差異。

本研究用壓片法測量這三個變種根細胞的染色體數量，並替大花咸豐草（花粉接受者）與另外兩變種（花粉提供者）進行人工授粉，以了解大花咸豐草的高度變異是否有可能是因為其與其它變種雜交的結果。結果發現：同一個變種的不同族群其染色體數量相同，大花咸豐草均為四倍體（ $4x = 48$ ），而小白花鬼針與白花鬼針為六倍體（ $6x = 72$ ）。在接受另外兩變種的花粉後，大花咸豐草並無成功結出瘦果。結果顯示大花咸豐草個體間許多特徵具有高度變異的現象，應該不是因為跟其它變種雜交的結果，比較可能是因為大花咸豐草具完全異交的繁育系統。

關鍵詞：菊科、咸豐草、染色體、雜交、入侵植物

Abstract

The three varieties of *Bidens pilosa* L. are not only different in their ray florets but also in breeding systems. Could it be possible that they have different chromosomal numbers? In addition, the heterogeneities of traits of var. *radiata* were higher than those of var. *minor* and var. *pilosa*.

In this study, I used squash method and counted chromosomal numbers of populations of these three varieties distributed in Taiwan. I also conducted hand pollination treatment between var. *radiata* (pollen receiver) and var. *minor* or var. *pilosa* (pollen donor) to understand whether the high variation in many traits in var. *radiata* resulted from hybridization with other varieties. No difference was found in chromosomal numbers of populations of the same variety. Forty-eight chromosomes and seventy-two chromosomes were counted for var. *radiata* and var. *minor* (and var. *pilosa*), respectively. Because the basic chromosome number for genus *Bidens* has been reported as $x = 12$. Accordingly, var. *radiata* is tetraploidy ($2n=48$), while var. *minor* and var. *pilosa* are hexaploidy ($2n=72$). No successful hybridization was observed between var. *radiata* and var. *minor* or var. *pilosa* in hand pollination experiment. Results suggested that highly variation in many traits among individuals of var. *radiata* is unlikely caused by hybridization with other varieties, but might be resulted from obligate xenogamous breeding system carried by the variety.

Keywords: Asteraceae, *Bidens pilosa*, Chromosomes, Crossing, Invasive plant

Introduction

Bidens pilosa L. (Hairy beggar-ticks, Asteraceae) is a cosmopolitan weed. With its center of diversification in Mexico, it is widely distributed in tropical and subtropical regions (Ballard, 1986). This taxon is an annual or perennial herb with square stems and opposite leaves and often occupies roadsides, disturbed sites and cultivated fields (Peng *et al.*, 1998). Many characters within this taxon are highly variable. For example, plants may be erect or decumbent; leaflets may be simple, tripartite, or dissected; capitula may be discoid or radiate, and the achenes may be awnless or 2 - 5 awned. According to the morphological characters, Sherff (1937) classified *Bidens pilosa* into six varieties: var. *pilosa*, var. *minor*, var. *radiata*, var. *bimucronata*, var. *calcicola*, and var. *alausensis*. Among these six varieties, var. *pilosa* and var. *minor* have wider distribution range than the other four varieties (Ballard, 1986).

Morphological characters are commonly used to assess the relationships among species. However, highly morphological variations not only occurred among different populations but also within a single population of *B. pilosa*. Therefore, Sherff's classification of *B. pilosa* is controversial because Sherff's taxonomic treatments were based primarily on herbarium specimens (Ballard, 1986). Chromosomal number and breeding system of 200 populations of *Bidens pilosa* in southern United States, Mexico and Central America have been investigated to clarify the classification of *Bidens pilosa* (Ballard, 1986). After the analyses, Ballard concluded that *B. pilosa* in North and Central America should be treated as a species complex containing three species with different chromosome number due to different ploidy: *Bidens odorata* ($2n=24$), *Bidens alba* ($2n=48$) and *Bidens pilosa* ($2n=72$). Species with different polyploidy also had different breeding system: hexaploid populations were self-fertile

while diploid and tetraploid populations were self-incompatible. According to Ballard's study, plants previously classified as var. *pilosa* and var. *minor* by Sherff's system still belonged to *B. pilosa*. But those being classified as var. *radiata* by Sherff should be treated as *B. odorata* (diploid, $2n=24$) or *B. alba* (tetraploid, $2n=48$), depending on their chromosome number.

The Flora of Taiwan listed three varieties of *Bidens pilosa* L. in Taiwan, var. *minor*, var. *pilosa* and var. *radiata* (Peng *et al.*, 1998). *B. pilosa* var. *radiata* was first recorded in Taiwan in 1984, and become one of the most noxious invasive plants in the past three decades (Wu *et al.*, 2004; Wu *et al.*, 2010). Thirty years ago, var. *minor* widely distributed in Taiwan. However, after var. *radiata* being introduced into Taiwan, var. *radiata* has become a serious invasive plant and dominant over the other two varieties within 30 years. The most obvious differences in morphology among these three varieties are their flowers. The capitula of var. *pilosa* are discoid (without ray florets), and the capitula of var. *minor* and var. *radiata* are radiate (with ray florets). *B. pilosa* var. *minor* and var. *radiata* both have 5-8 ray florets without androecium. The ray florets of var. *minor* are often shorter than 8 mm, while the ray florets of var. *radiata* are often longer than 10 mm. However, highly variation in ray florets size was found in var. *radiata*. In addition to their differences in ray florets, in a previous study, I also found that breeding system of these three varieties differed (Huang and Kao, 2014). *B. pilosa* var. *minor* and var. *pilosa* are self-compatible while var. *radiata* is self-incompatible. Based on Ballard's study, the three varieties of *B. pilosa* in Taiwan might have different chromosomal number. In addition to the highly variation in the size of its ray florets, significant variation in pollen/ovule ratio and disc floret number were also found in var. *radiata*. Because var. *radiata* is self-incompatible and sometimes growing sympatric with var. *minor* and var. *pilosa*,

could it be possible that the variety can hybridize with the other two varieties resulting in the highly variation in floral traits?

The objectives of this study were to understand (1) whether the three varieties have different chromosome numbers, and (2) whether the three varieties can hybridize. To achieve the goal, I counted chromosomal numbers of these three varieties and conducted hand pollination treatment between var. *radiata* and var. *minor* or var. *pilosa*.

Materials and Methods

Chromosome numbers

Achenes of each varieties of *Bidens pilosa* were collected from 5 - 8 populations distributed in Taiwan (**Figure 6-1**). After taken back to laboratory, achenes were planted in 1L pot for about one month. When plants reached 20 cm height, their shoots were cut and immersed in water for producing adventitious roots.

After adventitious roots growing 3 - 5 cm, 1 - 2 cm rootlets with root-tips were cut, pretreated with 2mM 8-hydroxyquinoline at 14°C for 5hrs, fixed in Carnoy's solution (95% EtOH: acetic acid = 3:1, v/v) for 24 hrs and then stored in 70% EtOH at -20°C. After washed in distilled water for two times, rootlets were hydrolyzed in 1N HCl at 60°C for 8 min and rinsed twice in distilled water again. In the following, materials were stained with leuco-basic fuchsin in the dark at room temperature for 2 hrs and treated in 1% pectinase at room temperature for 1 hr to separate the cells from each other. Root-tips were spread on a slide and squashed in a drop of 45% acetic acid and then observed under a microscope (Grombone-Guaratini *et al.*, 2006).



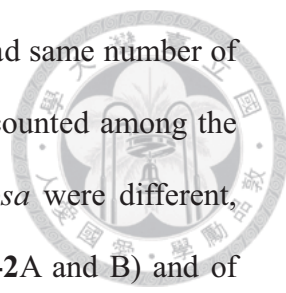
Hand-pollination

Achenes collected from populations in Chiayi, where the three varieties grew sympatric, were planted in pots and placed in the farm of National Taiwan University. After about two months, when these plants bloomed the flowers of these plants were used in crossing trials. Because var. *minor* and var. *pilosa* are highly self-compatible and self-pollinating (Huang and Kao, 2014), these two varieties were used as the pollen donors in crossing trials. In contrast, var. *radiata* being self-incompatible (Huang and Kao, 2014) was used as pollen receivers in crosses. In addition, crosses between individuals from different populations of var. *radiata* were also conducted as control. Plants growing in National Taiwan University and those growing in Chiayi populations were used as pollen donors and receivers, respectively. The inflorescence of *Bidens pilosa* was capitulum. Their hermaphroditic tubular florets opened in centripetal sequence, and the duration from first tubular floret blooming to last tubular floret blooming was about three to four days in each capitulum. Therefore, hand pollination was conducted once a day for three or four days during the period of anthesis. After receiving hand pollination, these capitula were wrapped in fine nylon-mesh netting bags (7×9 cm) to prevent the deposition of other pollen grains.

Results and Discussion

Chromosome numbers

The chromosomes of *B. pilosa* var. *radiata*, var. *minor* and var. *pilosa* were small (ca. 2 μ m in length) and shared similar morphology (with centromeres in the central region of the chromosomes) (**Figure 6-2**).



For each variety, plants collected from different populations had same number of chromosome. However, different numbers of chromosomes were counted among the three varieties. Although the capitula of var. *minor* and var. *pilosa* were different, chromosome numbers in plants of *B. pilosa* var. *minor* (**Figure 6-2A and B**) and of var. *pilosa* were both counted as $2n = 72$ (**Figure 6-2C and D**). Different from these two non-invasive varieties, the chromosome number of var. *radiata* was $2n = 48$ (**Figure 6-2E and F**).

Different level of ploidy is one of the most frequent reasons leading to difference in chromosomal numbers in some genera of Asteraceae (Solbrig *et al.*, 1972; Keil *et al.*, 1988; Dematteis and Fernandez, 2000). Thus, the variation in chromosomal numbers among the three varieties might result from polyploidy. The basic chromosome number for genus *Bidens* has been reported as $x=12$ (Ballard, 1986). Accordingly, *B. pilosa* var. *radiata* is tetraploidy, while var. *minor* and var. *pilosa* are hexaploidy. In our previous study comparing the breeding system of these three varieties, I found that var. *radiata* is self-incompatible, while var. *minor* and var. *pilosa* are self-compatible (Huang and Kao, 2014). Thus, the tetraploid variety, var. *radiata*, is self-incompatible, while the hexaploid varieties, var. *minor* and var. *pilosa*, are self-compatible. Similar results were also found in 200 populations of *Bidens pilosa* species complex in Central America, where the tetraploid populations were self-incompatible while the hexaploid populations were self-compatible (Ballard, 1986).

Recent studies showed that there is a link between polyploidy and invasiveness (Lowry and Lester, 2006; Treier *et al.*, 2009; Goralski *et al.*, 2014). Indeed, in this study I found that the invasive var. *radiata* is polyploidy. However, the non-invasive varieties are also polyploidy and have more ploidy number than the invasive one. In

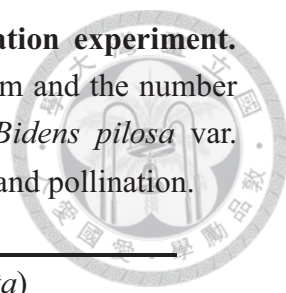
addition, their congener, *B. bipinnata*, which is not invasive in Taiwan, is also polyploidy ($2n = 72$) (Peng and Hsu, 1978). Thus, polyploidy alone cannot explain why var. *radiata* becomes invasive but not the other two varieties. There must be some other traits contributing to the invasiveness of var. *radiata* in Taiwan. The ability to reproduce vegetatively has been suggested as one of the traits (Hsu, 2006).

Hand-pollination

There were 32 - 57 disc florets in each capitulum of *B. pilosa* var. *radiata* (**Table 6-1**). In crossing trial, no viable achenes were produced by crossing between var. *radiata* and var. *minor* (or var. *pilosa*) or by hand pollination transferring pollen onto stigmas of the same individual of var. *radiata* (**Table 6-1**). In contrast, there were 17 - 29 mature achenes in capitulum of var. *radiata* receiving pollen from other individuals of the same variety. The result indicated that var. *radiata* is unlikely to hybridize with var. *minor* or var. *pilosa*. Accordingly, highly variation in floral traits found in *B. pilosa* var. *radiata* is unlikely caused by its hybridization with other varieties, but might result from the obligate xenogamous breeding system carried by this variety.

Table 6-1 Disc floret number and achene set in hand pollination experiment.

The sample size (n), number of disc floret per capitulum and the number of mature achenes per capitulum (means \pm SE) of *Bidens pilosa* var. *radiata* as a pollen receiver in crossing experiment by hand pollination.



Pollen donor	Pollen receiver (var. <i>radiata</i>)		
	n	Disc floret number	Mature achene
var. <i>minor</i>	3	48.7 \pm 3.4	0 \pm 0
var. <i>pilosa</i>	5	38.8 \pm 2.3	0 \pm 0
var. <i>radaita</i> (self)	4	31.8 \pm 4.5	0 \pm 0
var. <i>radiata</i> (cross)	6	42.3 \pm 3.2	23.0 \pm 2.2



Variety	Sampling site		
	Altitude (m)	Longitude (E)	Latitude (N)
<i>var. minor</i>	20	120°20'34"	23°11'15"
	1420	120°41'38"	23°30'21"
	1342	120°42'60"	23°28'41"
	2274	120°49'57"	23°28'56"
	18	121°35'21"	23°58'23"
	1156	121°22'47"	24°26'14"
<i>var. pilosa</i>	528	120°37'51"	23°26'03"
	2000	120°47'41"	23°29'57"
	26	121°34'53"	23°57'58"
	1255	121°22'23"	24°25'52"
	0	120°14'59"	23°29'44"
<i>var. radiata</i>	15	121°46'22"	24°40'39"
	149	120°41'0"	23°45'31"
	1420	120°41'38"	23°30'21"
	58	120°40'30"	24°07'26"
	20	120°23'52"	23°25'43"
	20	120°17'10"	22°55'29"
	20	120°17'39"	22°41'10"
	20	120°34'19"	22°36'49"

Figure 6-1 Sampling sites of three varieties. Achenes collected from six populations of *Bidens pilosa* var. *minor* (▲), five populations of var. *pilosa* (■), and eight populations of var. *radiata* (●). The altitude, longitude and latitude of these 19 sampling sites were also listed.

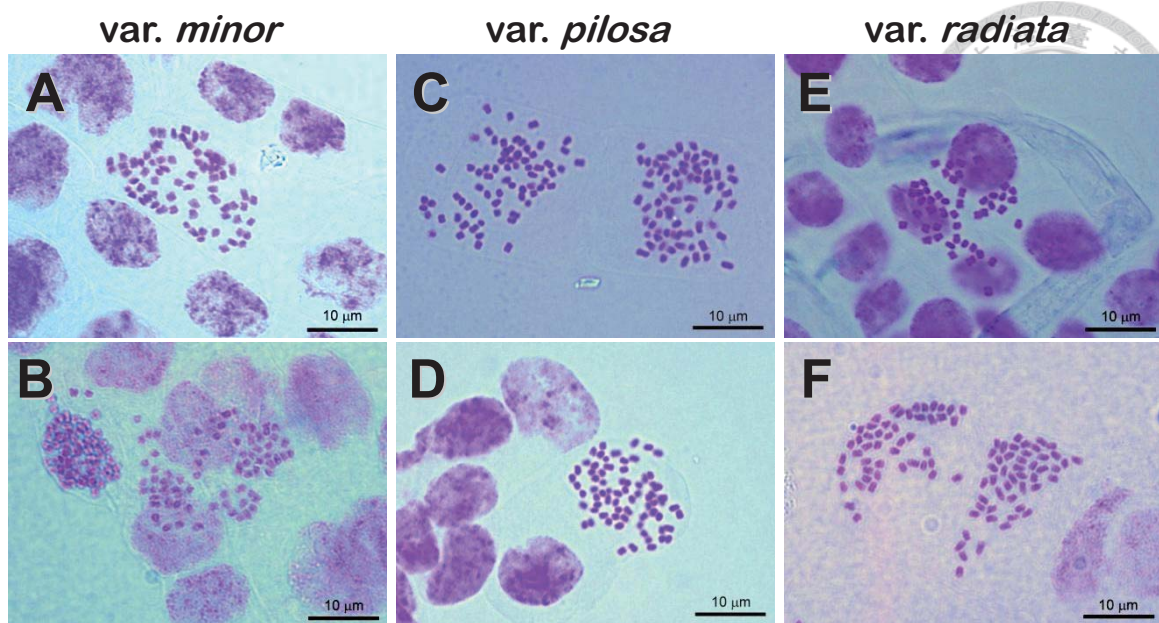


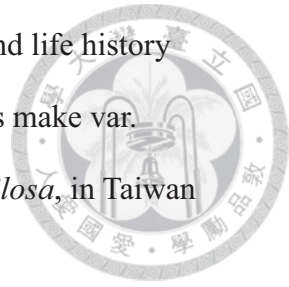
Figure 6-2 Somatic chromosomes of three varieties. Somatic chromosomes of two different populations of three varieties in *Bidnes pilosa*. (A) var. *minor* in Chiayi ($2n = 72$), (B) var. *minor* in Yilan ($2n = 72$), (C) var. *pilosa* in Chiayi ($2n = 72$), (D) var. *pilosa* in Hualien ($2n = 72$), (E) var. *radiata* in Nantou ($2n = 48$) and (F) var. *radiata* in Yilan ($2n = 48$) county in Taiwan.



Conclusions



In this dissertation, I compared the growth, breeding system, and life history traits of the three varieties of *Bidens pilosa* L. to evaluate what traits make var. *radiata* dominant over the other two varieties, var. *minor* and var. *pilosa*, in Taiwan lowland now.



Differences in (1) relative growth rate in summer; (2) breeding systems; (3) variance in traits among individuals; (4) the potential of vegetative reproduction; (5) life span; and (6) the numbers of achene production, were found between var. *radiata* and the other two varieties, var. *minor* and var. *pilosa*.

Growing faster than the other two varieties in summer makes var. *radiata* more competitive than var. *minor* and var. *pilosa* in the tropical and subtropical lowlands.

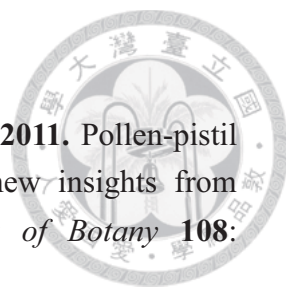
With **obligate xenogamous** breeding system, var. *radiata* has **higher variance** in many traits than var. *minor* and var. *pilosa*. The heterogeneities of traits might help var. *radiata* adapting heterogeneous and fluctuated environments, and then surviving in different habitats more easily than the other two varieties.

Having **higher potential of vegetative reproduction** and **longer life span** allows var. *radiata* to occupy the surrounding area more efficiently than var. *minor* and var. *pilosa*. In addition, with these two characters var. *radiata* is able to **set more achenes** and hence has more chance to be dispersed by animals than the other two varieties.

The differences in the aforementioned traits in combination with the warm climate and changes in land use pattern might contribute to the differences in the degree of invasiveness between var. *radiata* and the other two varieties in Taiwan lowland after the introduction of var. *radiata*. These might be also the traits that make *B. pilosa* var. *radiata* invasive in Taiwan.



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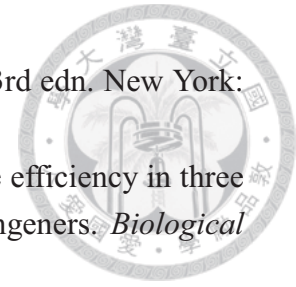
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