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Abstracts

Biological Control of *Rubus alceifolius* (Rosaceae) in La Réunion Island (Indian Ocean): From Investigations on the Plant to the Release of the Biological Control Agent *Cibdela janthina* (Argidae)

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Abstract

The giant bramble (*Rubus alceifolius* Poir.: Rosaceae), native to Southeast Asia, is one of the most invasive plants in La Réunion. A ten year research program was launched in 1997 with three components: i) genetic diversity, ii) development strategy, and iii) selection of biological control agents. Introduced populations in La Réunion, Mauritius, Mayotte and Australia were clonal and far from the highly variable native populations in Asia, while Madagascar populations appeared intermediate. Seed production is by apomixis in La Réunion Island and by allogamy in the native habitat. Fruit production occurs up to 1,100 m elevation while vegetative multiplication is possible up to 1,700 m. The plant grows in well lighted places, invading forest edges, and all open areas. From surveys of *Rubus* natural enemies in its native range, the sawfly *Cibdela janthina* (Klug) (Argidae) was selected as the most promising biological control agent and studied. The first population was thus released in La Réunion in early 2008 with the agreement of the local authorities for the biological control of *R. alceifolius*. It is now naturalized, spreading and under evaluation.

Introduction

Giant bramble (*Rubus alceifolius* Poir.: Rosaceae) is an invasive Southeast Asian bramble introduced to La Réunion Island (Indian Ocean) in the mid 19th century. In 1892, it was already cited as fatal for the island (Lavergne, 1978), and Rivals (1960) said "*it was a real pain for natural environment. Density was so high that regeneration of indigenous forest plants was impossible under Rubus thickets*." At that time, he also mentioned that "*only a biological solution could destroy this plant and solve the problem*".

Since the 16th century, the native ecosystems of La Réunion Island have undergone rapid transformation with the introduction of more than 2000 plant species, of which 628 have become naturalised (Lavergne et al., 1999). During their evaluation of the threat posed by invasive plants to the island, Macdonald et al. (1991) underlined that 62 exotic species were major threats, among them was *R. alceifolius*, which topped the list.

For many years, the *Office National des Forêts* attempted to bring the weed under control. But neither mechanical weeding nor chemical control was successful. Control was possible only on small surface areas, and needed to be repeated regularly. Also, the cost was extremely high (at present, €2 million is spent annually on the control of invasive plants) and the use of herbicides in native forests has raised ecotoxicological concerns. In 1992, the *Conseil Régional* of La Réunion made *R. alceifolius* a priority and decided to fund a research program to develop integrated control methods, with the emphasis placed on biological control. This program started in 1997 and ended in 2006 with the selection of a biological control agent. It was then followed by complementary action funded by the DIREN (Direction Régionale de l'Environnement) of La Réunion until 2009 for the introduction, acclimation and release of the selected biological control agent *Cibdela janthina* (Klug) (Hymenoptera: Argidae).

While much research has been done on the biological control of other species of *Rubus* (Bruzzese and Lane, 1996; Evans et al., 1999; Gardner et al., 1997; Groves et al., 1997; Julien and Griffiths, 1998; Nagata and Markin, 1986), little work has been done on tropical *Rubus* species or *R. alceifolius* itself. It concerned its distribution, seed production and spread in La Réunion (Sigala and Lavergne, 1996; Strasberg, 1995; Thebaud, 1989). The chrysomelid *Phaedon fulvescens* Weise, originating in Northern Vietnam, was mentioned as a potential candidate for biological control (Jolivet, 1984). Other natural enemies were an unidentified stick insect and two rust fungi, *Hamaspora acutissima* P.Syd. & Syd. and *Gerwasia rubi* Racib. recorded in Thailand by Taysum et al. in 1991 (unpublished document). All previous experiments carried out worldwide in the biological control of *Rubus* spp., used pathogens.

The project was built around three components. The first concerned the plant's genetic diversity: i) to compare the diversity between the area of introduction and the native range in order to match genetic and geographic origins of the invader, and ii) to determine the degree of specificity needed by biological control candidates and the opportunity for their use throughout the area of introduction. The second component was the biological study of the weed under La Réunion environmental conditions in order to highlight factors for growth, multiplication and spread. The third component aimed to select and study the biological control candidates suitable for introduction and release in La Réunion.

This paper presents a compilation of the project's main findings leading to the proposal for a biological control of the giant bramble in La Réunion using the sawfly *Cibdela janthina*.

Materials and Methods

La Réunion Island (2,512 km² in area) is a volcanic island in the Indian Ocean rising to 3,061 m a.s.l., with considerable climate variations. Rainfall ranges from 8,000 mm per year on the east coast to 500 mm per year on the lowland west coast (Robert, 1986). The island's vegetation comprises four main types that are determined by a combination of rainfall and temperature: semi xerophytic tropical forest, lowland humid tropical forest, mountain humid tropical forest and ericoid vegetation at high elevations (Cadet, 1977).

The giant bramble, *Rubus alceifolius* Poir. (Rosaceae, subgenus *Malachobatus*) was first described as a subspecies of *R. moluccanus* L., but is now considered a separate species (Kalkmann, 1993). It is a shrub with arching or climbing branches up to 5 m long in native areas or up to 15 m in length in areas of introduction. Branches and leaves bear curved prickles and yellow hairs. Stipules are large, orbicular, and deeply digitately divided. Leaves are simple, orbicular to broadly ovate, 10-30 cm in diameter, with 5-7 lobes, and cordate at the base. Inflorescence is terminal, consisting of up to 4 racemes with up to 8 flowers. Flower bud is globular, petals are orbicular, white. Collective fruit is globular, succulent, 1 cm in diameter and containing many red drupelets (Kalkmann, 1993). The giant bramble is exotic in Australia (Queensland), Mauritius, Mayotte, Madagascar and La Réunion Island but is native to Southeast Asia (southeast China, Hainan, Taiwan, Myanmar, Thailand, Laos, Cambodia, Vietnam, Indonesia/Sumatra, Java, Malaysia, Lesser Sunda Islands, Borneo and Sulawesi) (Friedmann, 1997; Kalkmann, 1993; Parsons and Cuthbertson, 1992). At La Réunion it frequently occurs as large stands along forest edges, road and river sides from sea level up to 1,700 m of elevation (Baret et al., 2004).

Genetic diversity studies of *R. alceifolius* were based on 224 specimens: 116 from introduced populations (La Réunion (75), Mayotte (8), Mauritius (7), Madagascar (19) and Australia/Queensland (7), and 108 from native populations (Thailand (59), Vietnam (30), Laos (1), Java (4), and Sumatra (14). Thirty specimens of other *Rubus* species were also evaluated (La Réunion (3), Thailand (12), Vietnam (10), Laos (2), and Sumatra (3). Plant DNA was extracted and processed following the protocol of Bousquet et al. (1990). Genetic differentiation was assessed by amplified fragment length polymorphism (AFLP), using 4 primer pairs and the restriction enzymes *Eco*RI and *Mse*I, as detailled by Amsellem et al. (2000). The genetic distance between individuals was calculated and expressed as the Simple Matching distance (Rohlf, 1993). A tree was constructed according to the Neighbour-Joining Method (Felsenstein, 1993). The study focused on two levels of comparison: individuals and areas (Amsellem et al., 2000).

The reproductive biology of *R. alceifolius* in the native and introduced habitats was assessed and compared using microsatellite markers specific for *Rubus* species (Amsellem et al., 2001a). We compared the reproductive system (fruit set) of *R. alceifolius* in its native range (nine half-sib seedlings derived from different fruits on a single plant sourced from Vietnam), in its area of introduction in Madagascar (three individuals and their half-sib progeny from two localities), and in La Réunion where 44 flowers from several parents were manually fertilized with pollen from other plants (Amsellem et al., 2001b).

Developmental patterns were then studied at La Réunion by conducting architectural and morphometric analyses that described individuals at the five specific growth stages from seedlings to mature plants (Baret et al., 2003a; Baret et al., 2003b). Altitudinal variations in fertility and vegetative growth were assessed by counting flowers, fruits, seeds and leaves in eight randomly located quadrats at six sites from 50 m to 1,500 m a.s.l., and by estimating the soil seed bank (Baret et al., 2004).

Biological control agents were selected from surveys carried out in the native range (Vietnam, Laos, Thailand, China, Indonesia (Sumatra) and Singapore) from 1997 to 2004, and in the area of introduction at La Réunion Island (1998). Altogether, stands of *Rubus* were examined at 309 different locations subject to different climatic conditions, ranging from the lowlands to the highest elevations (2,500 m a.s.l. in Doi Inthanon Mountains, Northern Thailand) and from equatorial (Toba Lake, Northern Sumatra) to tropical climates with fairly cold winters (Guangdong and Hainan mountains in China). *Rubus* natural enemies (arthropods or pathogens) were collected and identified. Symptoms and environmental conditions were recorded. The most promising ones were collected alive and reared in laboratories in Sumatra, Montpellier and La Réunion for further biological and specificity studies in order to select those most suitable for biological control of *R. alceifolius* at La Réunion.

Results

Rubus genetic diversity and putative origins of *R. alceifolius*

The genetic study of *R. alceifolius* revealed two well-separated groups, both distinct from the out-group corresponding to other *Rubus* species. The first group consisted of all the samples taken within the native range and the second group, all the samples from the area of introduction. A study of the within-area diversity showed relatively marked genetic diversity between individuals from countries of the native range. On contrary, each population sampled in the Indian Ocean islands (La Réunion, Mayotte, Mauritius, and Australia), with the exception of Madagascar, was characterized by a single different *R. alceifolius* genotype. All these genotypes were closely related to individuals from Madagascar where polymorphism was intermediate between the situation in areas of introduction and the native range.

Many authors have discussed the different hypothetical origins of this exotic plant. E. Jacob de Cordemoy (1895) mentioned that it may have been introduced at La Réunion in 1846. Nevertheless a previous text "*Ralliement du 17 août 1892*" cited by Lavergne (1978) specified that this plant came from the Botanical Garden in Calcutta. Other authors thought it was sent from Vietnam (Jolivet, 1984), or that it may either have been introduced first in Mauritius by Commerson on his return from Java in 1768 (Rivals, 1960), or introduced into Mauritius from Madagascar (Vaughan, 1937) and then spread to La Réunion in the 1850s (Rivals, 1960). Our results suggest that *R. alceifolius* was first introduced into Madagascar, perhaps on multiple occasions. The Madagascan individuals were thus the immediate source of the plants that colonized other areas of introduction. Successive nested founder events appear to have resulted in the populations in the area of introduction showing a cumulative reduction in genetic diversity. The marked genetic differences between the populations in the native range and in the area of introduction prevented us from determining the geographical origin of the alien plant (Amsellem et al., 2000).

Genetic diversity and the weed's biological strategy as pointers for control

The low diversity of the populations found in most areas of introduction suggested that a biological control agent efficient against one individual should be able to attack the entire population on the island.

Both the genetic diversity patterns and differences between half-sib progeny and their maternal parents (revealed by microsatellite markers) showed that in the native range, seeds are produced sexually (Amsellem et al., 2001a; Amsellem et al., 2001b. By contrast, in Madagascar, over 85% of the half-sib progeny resulting from open pollination gave multilocus genotypes identical to those of their respective maternal parents. Seeds thus appear to be produced mostly or exclusively by apomixis in Madagascar. We therefore suggest that Madagascan populations resulted from the hybridization of an introduced *R. alceifolius* and native populations of presumably *R. roridus* Lindley that Kalkman (1993) considered similar and synonymous to *R. alceifolius.* Apomixis was therefore a consequence of this hybridization. In Reunionese populations of *R. alceifolius*, seeds obtained in controlled pollination experiments were all genetically identical to the maternal parents. While genetic variation (microsatellite markers) in Reunionese populations was low, it was sufficient to demonstrate that seeds could not have resulted from fertilization by the pollen donors chosen for controlled pollinations, or from autogamy, but were produced exclusively by apomixis (Amsellem et al., 2001b). This phenomenon was responsible for the clonal population in the area of introduction.

The architectural and morphological studies showed five developmental stages for *R. alceifolius,* differing by several markers such as internode length and diameter, pith diameter, and plant shape. A heteroblastic developmental pattern was thus revealed for the plant, midway between that of a bush and a vine. The results also showed that this species taps environmental resources early in its development, whereas it "explores" the environment during the adult stage (Baret et al., 2003b).

To determine the invasive capacity of *R. alceifolius*, fertility and vegetative growth were studied at different altitudes on La Réunion Island (Baret et al., 2005). Flowering period duration, seed production, and the seed bank were found to be negatively correlated with elevation (50 – 1,500 m a.s.l.). At a lowland site, fruit production averaged between 30 and 80 fuits m-^{2}, while no fruits were observed above 1,100 m. The seed bank was greater under *R. alceifolius* patches (>10,000 seeds.m-^{2}) than in understories not colonized by the bramble (approximately 3,000 seeds.m-^{2}). Seed dispersion in forest was mainly by running water. Although the number of leaves per unit area was similar along the entire gradient studied, the reduced fruiting in upland areas might be offset by an increase in vegetative growth. Monospecific bramble patches in lowland areas may serve as the sources of seed for the colonization of new areas by bird dissemination. Once established at high elevations, the weed grows vegetatively without flowering and multiply by layering, cutting or sucker (Baret et al., 2004).

Selection of potential biological control agents

Fifty one arthropods and four pathogens were recorded and collected during the surveys conducted in the native range and in La Réunion Island. Particular care was taken to select agents on the basis of a combination of criteria (type of damage to and impact on *R. alceifolius*, host specificity, life traits etc.) for further biological and specificity studies. Of the leaf feeders, sawflies from Sumatra and China (*Cibdela janthina, C. chinensis* Rohwer, and *Arge siluncula* Konow) appeared to be the most promising. They caused complete defoliation of the weed and seemed to be highly specific. The beetles *Phaedon fulvescens* and *Cleorina modiglianii* Jacoby, found respectively in Vietnam and Sumatra, were also promising. The rust fungus *Hamaspora acutissima* was observed and collected in many places throughout Asia. Only a few common insects were reported in La Réunion Island, but never damaging *R. alceifolius*.

Phaedon fulvescens Weise (Coleoptera: Chrysomelidae) was collected in Northern Vietnam while feeding on *Rubus* spp. leaves on the edge of mountain forests at 900 m a.s.l. Field observations coupled with biological and host specificity studies in Montpellier generated a wealth of new information about this species. Although not encountered frequently, individuals were very numerous in the populations observed and both adults and larvae damaged the plant by leaf skeletonizing. Eggs were laid separately on the underside of leaves and coated with feces. We also noted that the insect undergoes a two-month summer diapause. Therefore, this insect may only complete a single generation in a year, not three or four as initially thought. Field observations indicated that the beetle was highly specific to *Rubus* species of the *Malachobatus* subgenus. But host specificity tests carried out on *R. apetalus* (indigenous of La Réunion) of the *Ideobatus* subgenus showed that the insect can also feed on this plant and survive throughout its lifecycle. We therefore rejected *P. fulvescens* for the biological control of *R. alceifolius* in La Réunion (Le Bourgeois et al., 2004).

Cleorina modiglianii Jacoby (Coleoptera: Chrysomelidae) was found in Sumatra on several *Rubus* spp. in the shade provided by *Pinus merkusii* Jungh. & de Vriese forests from 700 to 1,200 m. This beetle caused leaf skeletonizing damage to *R. alceifolius* and *R. moluccanus* L. We were unable to find eggs or larvae despite numerous field surveys. Host specificity tests were carried on adults in the laboratory, comparing *R. alceifolius* (Réunion), *R. alceifolius* (Sumatra), *R. apetalus* (L Réunion) and *R. fraxinifolius* (La Réunion). The adults only fed and survived on *R. alceifolius* (Sumatra) and *R. apetalus* (La Réunion). This insect was therefore rejected as a potential candidate for biological control.

Cibdela janthina (Klug) (Hymenoptera: Argidae) was recorded in Sumatra. The insect's behavior was observed in the field while its biological traits were assessed in the laboratory in Sumatra. Mating happened in full light at 30°C and 80% humidity two days after female emergence. Then eggs were inserted into the main nerves of the plant's upper young leaves not yet fully opened. Average fertility was 58 eggs per female, with 84% viability. The eggs hatched after 10 days of incubation. The larvae completed seven instars within 25 to 30 days, and then pupated in a silk cocoon under the leaf litter. Larvae were gregarious during the major part of their development and presented a typical S-shape. The full life cycle ranged from 48 to 62 days. The insect may complete six generations per year without any diapause. Adult life span was only 7-14 days and they were found not to be feeding under Sumatran environmental conditions; only drinking dewdrops on leaves, while the larvae were feeding on *R. alceifolius* leaves consumed systematically along the branches in a top-down process. Host range tests were conducted on 41 plant species from 13 botanical families chosen on the basis of phylogeny and economic or conservation issues for La Réunion. *Cibdela janthina* appeared to have a very narrow host range, feeding only on *Rubus* species. Starvation tests showed some feeding on certain subspecies of *R. moluccanus* not present in La Réunion, on *R. fraxinifolius* (exotic to La Réunion) and on *R. apetalus* (indigenous in La Réunion). Choice tests showed that the insect mainly prefer to feed on *R. alceifolius*. Temperature conditions that impact on the insect's development should keep *C. janthina* under 1,000 m of elevation while *R. apetalus* is present from 700 m to 1,700 m. Considering these results and the insect's biological features, *C. janthina* was considered as a good potential biological control agent against *R. alceifolius* in La Réunion. Accordingly, a petition was made for permission to introduce and release it.

Hamaspora acutissima P.Syd. & Syd. (Uredinales: Phragmidiaceae) was observed at many locations in Vietnam, Thailand and Indonesia (Sumatra). It was visible on the upper face of leaves as small brown to yellowish spots and on the lower face as bunches of orange paraphyses containing teliospores. Spots were found on isolated leaves and plants, or as intense infestations covering all parts of *Rubus* plants. In cases of marked contamination, leaves were drying and withering. All the *Rubus* mentioned as infected by this fungus belonged to the *Malachobatus* subgenus indicating the narrow host range of the rust. Biological and host specificity studies showed that Asian *Rubus* species belonging to the *Malachobatus* subgenus could sometimes be inoculated. *R. alceifolius* from La Réunion was inoculated but the pathogen stopped growing at the mycelium stage without producing new teliospores. Rust fungi are known to be highly specific (Evans and Gomez, 2004). Our results confirmed that the Reunionese *R. alceifolius* is genetically too different from those in the native range to allow *H. acutissima* attack.

Conclusion

The alien invasive giant bramble found in La Réunion and other areas of introduction is genetically different from those in the native range and probably resulted from hybridization in Madagascar. It has a high growth potential and produces apomyctic fruits in the area of introduction. With its marked phenotypic plasticity, the plant is able to fruit below 1,100 m a.s.l. and can grow and multiply vegetatively up to 1,700 m.

Of the biological agents collected in the bramble's native range, the sawfly *C. janthina* showed the best biological and ecological traits and host specificity that could justify its introduction into La Réunion to regulate *R. alceifolius* populations under 1000 m a.s.l. It also appeared to have the most severe impact on weed growth.

Therefore, a petition form to introduction and release of *C. janthina* was submited in 2006 to the *ad hoc* scientific committee and was accepted by local authorities. Cocoons from Indonesia/Sumatra were introduced in mid 2007 for the rearing of the sawfly and acclimatization at La Réunion. The first population was released on the east coast of La Réunion in early 2008. It is now spreading well, and controlling populations of the giant bramble. Studies of the spread dynamics and impact of *C. janthina* on *R. alceifolius* thickets at the island level are ongoing, as is a study of the impact of the decline of populations of the giant bramble on natural vegetation dynamics.

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