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Assessing the suitability and safety of a well-known bud-galling wasp, *Trichilogaster acaciaelongifoliae*, for biological control of *Acacia longifolia* in Portugal

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Abstract

Acacia longifolia is a widespread invasive plant species in Portugal. In South Africa, it is controlled by a bud-galling wasp, *Trichilogaster acaciaelongifoliae*, which could also be used in Portugal. Biological control of invasive alien plants has received little consideration anywhere in Europe and has never been attempted in Portugal. The lack of a suitably-large quarantine facility necessitated the use of a novel approach to test non-target species in Portugal. Mature *T. acaciaelongifoliae* galls were shipped to Portugal from South Africa to obtain adult female wasps which were confined in Petri dishes each with a bud-bearing branch of one of 40 non-target plant species. The time spent by the wasps exploring and probing the buds was measured after which buds were dissected to detect any egg deposition. The results showed that *T. acaciaelongifoliae* did not respond to the buds of most (23) species. The females spent time on the buds of the other 17 species but only laid eggs in three species besides *A. longifolia*. Oviposition on *A. melanoxylon* was expected but was not anticipated on *Vitis vinifera*, vines, (where eggs were deposited externally in the pubescent coat of the buds) or on *Cytisus striatus*, broom, (where eggs were inserted into the buds as they are on *A. longifolia*). Subsequent trials on potted plants showed that galls only developed on *A. longifolia*. Field surveys in South Africa and Australia showed that galls never occur on either vines or broom. The implications of these findings for the use of *T. acaciaelongifoliae* for biological control of *A. longifolia* in Portugal are considered in relation to the wealth of experience and knowledge about the specificity of the wasp and the reliability of conducting host-specificity tests under confined conditions of cages.

Key words: *Acacia longifolia*, biocontrol, buds dissection, Europe, invasive plant species, specificity tests, Sydney golden wattle, *Trichilogaster acaciaelongifoliae*.

1. Introduction

Acacia longifolia (Andrews) Willd. (Sydney golden wattle, long-leaved wattle) is a small leguminous tree or shrub, native to south-eastern Australia, which is invasive both in Portugal (Marchante, 2001; Marchante et al., 2003) and South Africa (Dennill et al., 1999), as well as other regions of the globe (Elorza et al., 2004). Some authors (Whibley, 1980) recognize two subspecies within this taxon: *A. longifolia* (Andrews) Willd. subsp. *longifolia* and *A. longifolia* (Andrews) Willd. subsp. *sophorae* (Labill.) Court., whereas others treat *A. longifolia* and *A. sophorae* (Labill.) R. Br. as distinct species (Paiva, 1999). *Acacia floribunda* (Vent.) Willd is closely related to *A. longifolia*, and in the past, it was considered a subspecies of *A. longifolia* (Maslin, 2001).

Acacia longifolia was first introduced to Portugal in the late 19th and early 20th century to curb sand erosion along coastal dunes (Neto, 1993). Since then *A. longifolia* has spread into other areas, both naturally and by horticulturists who favor its bright yellow flowers (Almeida, 1999). It grows rapidly and has prolific production of seeds which accumulate in the soil, reaching levels of 1500 seeds/m^2 (Marchante et al., 2010b).The seeds respond to fire and germinate *en masse* in the ash beds (Pieterse and Cairns, 1988). With time, extensive thickets have formed in coastal sand dunes and a variety of other habitats, particularly along rivers, road edges and on mountain slopes (Marchante et al., 2008a). *Acacia longifolia* is legally considered as an invasive species in Portugal whose use is prohibited (Ministério do Ambiente, 1999). Its ability to fix nitrogen (Rodríguez-Echeverría et al., 2007), and the absence of natural enemies, contribute to making *A. longifolia* a highly competitive species capable of shading out native species (Marchante et al., 2003) and posing a substantial threat to local biodiversity (Marchante, 2001) while changing soil properties and altering ecosystems processes (Marchante et al., 2008b; Marchante et al., 2009; Marchante et al., 2008c).

In Portugal, control of *A. longifolia* relies on mechanical methods, mainly basal cutting and, to a less extent, on chemical application of herbicide to the cut ends of the stumps. These methods are prohibitively expensive and have failed to achieve lasting control (Marchante et al., 2004), mostly due to replenishment of thickets from the abundant seed banks in the soil (Marchante et al., 2010a).

In South Africa, where *A. longifolia* had been problematic for over a hundred years, biological control with an Australian gall wasp, *Trichilogaster acaciaelongifoliae* Froggatt (Hymenoptera: Pteromalidae), later assisted by a seed-feeding weevil *Melanterius ventralis* Lea (Coleoptera: Curculionidae), has proven to be an excellent management option (Dennill, 1988 ; Impson and Moran, 2003) which is not yet available in Portugal.

The first *T. acaciaelongifoliae* individuals introduced to South Africa, during 1982, were collected in Australia from its two known, closely-related hosts, *A. longifolia* and *A. floribunda* (Dennill, 1987). Soon after release, it became clear that besides having a direct effect on seed production (Dennill, 1990; Dennill et al., 1999), *T. acaciaelongifoliae* galls also act as nutrient sinks and thereby indirectly inhibit the development of both reproductive and vegetative growth of their host plants, often causing die back of branches and whole plants when environmental conditions are harsh (Dennill, 1985; Impson and Moran, 2003). There have been no quantitative surveys to demonstrate the overall effectiveness of the wasp as a biocontrol agent (Hoffmann et al., 2002) but *A. longifolia* is generally no longer considered to be anywhere near as problematic as it was formerly, a change that is attributed to biological control having succeeded in South Africa.

Recently, consideration has been given to using *T. acaciaelongifoliae* in Portugal for biological control of *A. longifolia*. The prospects of success are good because the wasp has a proven track record and because it is highly host specific. The specificity of *T. acaciaelongifoliae* was confirmed before it was released in South Africa by exposing potted plants to the insects under caged quarantine conditions and subsequently seeking signs of gall development (Dennill et al., 1993; Van den Berg, 1980). Since its release, *T. acaciaelongifoliae* only utilizes two of its known Australian host plants, *A. longifolia* and *A. floribunda* (Dennill and Donnelly, 1991; McGeoch and Wossler, 2000). Underdeveloped galls are very rarely seen on *Paraserianthes lophantha* (Willd.) Nielsen and *Acacia melanoxylon* R. Br. in South Africa, but only when these plants occur in close proximity to heavily galled *A. longifolia* plants and neither of the two species is considered to be a suitable host for the wasp (Dennill et al., 1993). The host specificity of *T. acaciaelongifoliae* is not unexpected because insects that attack and live within the reproductive parts of their host plant (especially gall-forming insects) almost always display a high degree of monophagy (Ananthakrishnan, 1984).

Although classical biological control has been used against insect pests in Europe (EPPO, 2008), to date only one biological control agent has been released against an alien invasive plant (EPPO, 2010; Shaw et al., 2009; Sheppard et al., 2006). Despite the unequivocal body of evidence that *T. acaciaelongifoliae* is highly host specific (Dennill et al., 1993), due to the novelty of the process in Europe, regulatory authorities in Portugal insisted that additional evidence should be obtained to confirm that *T. acaciaelongifoliae* will not inflict any damage on non-target hosts, noting that the insects would encounter a distinctive suite of plants in a different hemisphere.

The lack of a suitably-large quarantine facility to perform host specificity tests presented a challenge in determining how the wasps would respond to the plant species on the list that was drawn up. This paper describes the methods that were used, the results that were obtained and the implications of the findings for deciding whether or not *T. acaciaelongifoliae* should be cleared for release in Portugal.

2. Materials and methods

2.1. Biology of agent and host plant

The biology *of T. acaciaelongifoliae* has been described by Noble (1940) and Dennill (1985; 1987). It is a small, parthenogenetic, univoltine bud-galling wasp. In the southern hemisphere, *T. acaciaelongifoliae* adults emerge predominantly in spring and early summer (October to December) and immediately commence oviposition (Dennill, 1987). The eggs lie dormant until late winter when they hatch and multilocular galls start to develop. Each larva has a discrete chamber in which it completes its development. Most chambers contain females but occasional males develop in smaller chambers on the periphery of the gall.

Milton and Moll (1982) studied the phenology of *A. longifolia* in South Africa and showed that while the timing of events varies with habitat and location, there is active vegetative growth on the plants from September to December (spring and early summer) and then again in autumn (April-May). The flower buds are set as the new growth forms but flowers only develop between August and November after a period of bud dormancy in winter. Pods develop from September to November and are fully ripe by mid-November. The period when young buds are suitable for *T. acaciaelongifoliae* oviposition overlaps with the period when galls are maturing and pods are ripening. In Portugal, the phenology of *A. longifolia* also varies in the different regions where the plants occur and shows some seasonal differences to the southern hemisphere (C. Morais, unpublished data). Usually, first flowers are observed in December but full bloom is in February - March (late winter through to the beginning of spring). Pods develop from March to July and ripen between June and August. Small buds (1-3 mm) dominate in June-August but are still present in lower numbers until December. Vegetative growth occurs predominantly from April to August (spring and summer) (Morais and Freitas, 2008). Trees in more northern regions show a slightly delayed cycle.

2.2. Specificity test plant list

The plant species to be included in non-choice tests were selected according to criteria outlined by Briese (2002) and Briese and Walker (2002), including phylogenetic proximity and morphological similarity (specifically bud structure) to *A. longifolia*. Other factors considered were economic value, conservation importance (*e.g.,* endemic species), and biogeographic and ecological overlap (*i.e.,* plants that are common in sand dunes, the habitat most frequently invaded by *A. longifolia*). The selection included 40 species (Table 1) that fulfilled either one (*e.g.*, *Quercus faginea* Lam.) or several (*e.g.*, *Stauracanthus genistoides* (Brot.) Samp.) of the selection criteria. The final plant list was approved independently by ICNB (Portuguese Institute for Nature & Biodiversity Conservation), who had nominated some of the species on the list.

The degree of phylogenetic separation between the listed plants and *A. longifolia* was established following Judd et al. (1999), mainly to determine higher level of phylogeny (families, orders and major clades). Congeneric species were not included in the test list, with the exception of *A. melanoxylon*, because: a) there are no congeneric native species (or any other Mimosoideae) in Portugal or elsewhere in Western Europe; b) none of the introduced *Acacia* spp. has major economic value in Portugal; and c) several *Acacia* species (*A. baileyana* F. Muell.; *A. cyclops* A.Cunn. ex G.Don; *A. dealbata* Link; *A. decurrens* (J.C. Wendl.) Willd.; *A. floribunda* (Vent.) Willd; *A. mearnsii* De Wild; *A. melanoxylon* and *A. saligna* (Labill.) H.L. Wendl.) were subject to host specificity tests in South Africa where galls only developed on *A. floribunda*, a recognized host plant of *T. acaciaelongifoliae* in its native range, besides *A. longifolia. Acacia melanoxylon* was included in the tests to confirm the status of infrequent observations of sporadic gall formation on this plant species in South Africa.

The test species were separated into six categories on the basis of their phenology. The groups comprised the target weed *A. longifolia*, and five clades with increasing phylogenetic distance from the target weed (see Fig. 1), including: 1) species from the genus *Acacia*; 2) species from other genera within the family Fabaceae; 3) species from other families within the Order Fabales namely Polygalaceae; 4) species from more distant related families within the Rosidae (specifically clade Eurosids I, which includes the Fabaceae), namely Rosaceae, Salicaceae, Rhamnaceae, Ulmaceae, Fagaceae and Myricaceae; and 5) species from distant families outside the Eurosids I. Although some authors (Heywood, 1993; Izco et al., 1998) consider the Order Fabales to be monophyletic, including Fabaceae alone, others (Judd et al., 1999) recognize three families in the order, based on morphological characters and rbcL sequences, with the Polygalaceae being the only family with species present in Portugal.

Three annual species (*Vicia faba* L., *Pisum sativum* L. and *Phaseolus vulgaris* L.) were included on the list even though the wasp needs an entire year to complete its development within its gall, a mismatch which will preclude this group of plants as possible hosts. The three species where included because they belong to the same family as *A. longifolia* and because of their importance as economic crops.

2.3. Host specificity testing

2.3.1. No-choice tests – exploring of buds and oviposition by T. acaciaelongifoliae

Trichilogaster acaciaelongifoliae galls were collected from late September to December during 2005, 2006, 2007 and 2008 on the campus of the University of Cape Town, South Africa (33º57'S 18º27'E). For shipment, batches of galls were packaged in sealed polyester cloth bags inside cardboard containers which were air freighted to Portugal. The packaging allowed exchange of respiratory gases while ensuring containment of any insects that emerged in transit. The galls were received at Escola Superior Agrária de Coimbra (Portugal), where they were kept in a quarantine facility at approximately 25 ºC, 12: 12 L: D, conditions which were maintained before and during experiments.

The relative acceptability of all non-target plant species as oviposition sites for *T. acaciaelongifoliae* was assessed in no-choice tests with *A. longifolia* as a control. *Acacia longifolia* was collected from several localities in Coimbra (40º20'N, 8º40'W) and S. Jacinto Dunes (40º39'N, 8º44'W). Branches of test plants were collected immediately before initiation of the test and were transported with the cut end of the stem in a container of water to prevent wilting. In the laboratory, shoots containing small buds had the cut end covered with damp tissue paper which was held in place with aluminum foil. Each shoot was placed in a Petri dish (5 cm highl, 23 cm diameter) and exposed to one female wasp for the duration of its adult life (2 to 3 days). Nine branches were tested per species, each with a separate wasp in an individual cage. Whenever possible, each cage contained plants with comparable amounts of foliage and numbers of buds (frequently, seven or more buds). Some exceptions were inevitable due to distinct plant morphology, namely species with high numbers of small buds in close proximity to each other along the shoot (*e.g.*, *Erica scoparia* L. and *Corema album* (L.) D. Don) or species with buds widely spaced along the shoot (*e.g.*, *Ceratonia siliqua* L. and *Pinus pinaster* Aiton).

Each branch was characterized according to bud size (< 1 mm; 1 mm; 1.5 mm; 2 mm; ≥ 3 mm) and was presented to *T. acaciaelongifoliae* females to determine which buds were selected for oviposition. Exploring and probing of buds by each wasp was observed during nine observation sessions of 1200 sec (20 min) for each plant species. An observation session commenced when the wasp first moved on to the plant or after 5 min if this had not happened by then. Over the 4 years of the trials, there were 123 h of observations of the wasps.

2.3.2. Dissection of buds to detect T. acaciaelongifoliae *eggs*

After exposure to the female wasps, buds were dissected under a binocular microscope to determine the number of *T. acaciaelongifoliae* eggs that had been deposited, if any. At least seven buds (exceptionally less in species with fewer buds per mm of shoot) were dissected per branch. Eggs of *T. acaciaelongifoliae* are minuscule (approximately 0.2 mm in length), brilliant white and recognizable by their oval to oblanceolate shape.

2.3.3. Gall induction on potted plants

Plant species in which eggs were detected in buds were subsequently tested further, except for *A. melanoxylon*. This species was not included because it was particularly difficult to get the small potted plants needed for the experiment and it is already known to support gall formation sporadically, *i.e*., observations in the field in South Africa confirmed the result of the oviposition test. For each species, six small (30-90 cm) potted plants were enclosed separately in a plastic bag into which two adult *T. acaciaelongifoliae* females were added and left until they died, corresponding to ca. 2 days of contact. Wasps were transferred to the potted plant within 14 h of emergence. Two days after all the wasps had died, their remains were removed, the plastic bag was detached and the plants were moved outdoors and monitored for 6 months to detect whether or not there was any gall development. Given that the immature stages are endophagous and immobile there was no risk from moving plants outdoors during this phase of the life cycle. The numbers of galls formed, along with their dimensions, were recorded.

2.3.4. Surveys in South Africa and Australia

When possible, each of the species, or close relatives thereof, on which eggs were laid in quarantine was surveyed to determine whether the wasps induced galls on these species in South Africa and Australia. In South Africa, plants were surveyed in the Western Cape, Cape Region, where *A. longifolia* used to be very abundant and still exists at much lower levels. *Vitis vinifera* L. is widely cultivated in the region and *A. melanoxylon* is common but

Cytisus striatus (Hill) Rothm is not present at all. Another species of a former *Cytisus* (*C. monspessulanus* L. = *Teline monspessulana* (L.) K. Koch.) and the closely related *Spartium junceum* L. (Spanish broom) were surveyed for galls. For each plant species, sites were selected where the plant species being surveyed was growing in close proximity (< 25 m) to *A. longifolia* plants with galls. In Australia, plants were surveyed in New South Wales, Wollongong. *Acacia longifolia, A. melanoxylon, T. monspessulana* and *V. vinifera* were all surveyed but only *A. melanoxylon* was found in close proximity to *A. longifolia*.

For each sample, 10 plants of the test species and 10 *A. longifolia* plants were randomly selected and observations were made to determine whether the plants had galls by searching for at least 15 min. In species where galls were located, 10 branches were randomly selected and the terminal 70 cm of each was examined to record the number of *T. acaciaelongifoliae* galls per branch. The observations were made during November (2008 and 2009) and March (2009), when the galls were completely formed and easy to detect (both during and after emergence of the adult wasps).

2.4. *Statistical analyses*

Time spent on oviposition or exploring buds was recorded in seconds and mean values per species were calculated and compared between species using a General Liner Model (GLM), with a between-subject design One-way ANOVA. The bud dissections were used to calculate the percentage of both buds and branches with eggs for each plant species. The quantity of eggs per branch was also recorded and compared using One-way ANOVA. The buds were categorized according to size, and the mean number of eggs laid on each bud category on each plant species, was compared using a GLM with a between-subject design Factorial ANOVA. Differences between means were compared with Tukey's test at 5% level of significance. STATISTICA 6.0 (StatSoft, Inc. 2001, www.statsoft.com) was used for the statistical analysis.

3. Results

3.1. No-choice tests – observations of the oviposition behavior of T. acaciaelongifoliae

The wasps were observed exploring the buds of only 17 species (nine species had wasps stationary on the buds and 12 species had wasps that were active on the buds), with no significant differences between species (Table 2). Ovipositional probing was noted on the target species *A. longifolia* and six non-target species, including all the species where eggs were later detected. With the exception of *A. longifolia, A. melanoxylon* and *C. striatus,* this behavior was observed only once on each of the plant species.

Wasps were observed on the buds for ca. 3% of the total time of observations on all of the plants (123 h). On species where egg deposition was confirmed (Fig. 1), the wasps spent more time on the buds especially on buds of *A. longifolia, A. melanoxylon* and *C. striatus*.

3.2. No-choice tests – dissection of plant species buds to detect T. acaciaelongifoliae *eggs*

Dissection of the buds of *E. scoparia*, *Q. faginea* and *L. nobilis* L., all in the taxonomic Order Fabales (Fig. 1), showed that although the wasps had been observed probing the buds of these species (Table 2), no eggs were laid on any of these plants. Of the nine females placed on each plant species, seven laid eggs on the target species, *A. longifolia*, five laid on *A. melanoxylon*, four on *C. striatus* and two laid eggs on *V. vinifera*. Eggs were laid on 21.8% of the buds of *C. striatus* that were exposed to the wasps while on *A. melanoxylon* only about 10% of buds had eggs (Fig. 1). On *V. vinifera,* only 4.3% of the buds had eggs whereas on *A. longifolia,* eggs were laid in 31.8% of the buds. On *C. striatus* and *A. melanoxylon* (which were included in the test-list because of their close relationship to *A. longifolia*), eggs were laid within the bud tissues as happens on *A. longifolia*. In the case of *V. vinifera* eggs were laid on the protective, pubescent outer layer of the buds and not within the bud tissues.

The number of eggs per branch varied with plant species ($F_{3,32} = 4.182$, $p = 0.013$), with significantly more eggs laid per branch on *A. longifolia* than on *V. vinifera* and *A. melanoxylon* while the numbers laid on *C. striatus* were intermittent among *A. longifolia* and the other two species and not significantly different from any of the others (Fig. 2).

Trichilogaster acaciaelongifoliae showed a clear ‘preference’ for laying eggs on buds that were smaller than 3 mm (Fig. 3). On *C. striatus* most eggs were found in the smallest buds (<1-1.5 mm) while on *V. vinifera* the eggs were found predominantly on larger buds (1.5 - 2 mm). The target species *A. longifolia* had eggs in a wider range of bud sizes up to 3 mm with uniform pattern of around 30 and 40% of the buds in each size class having eggs. On *A. melanoxylon* the eggs were found mostly in the intermediate sized buds (1-2 mm). The pattern of bud use was also reflected in the numbers of eggs which were deposited in the different sizes of buds (Fig. 4). For each of the four plant species, buds generally decreased in size from the proximal to the terminal portion of the branches.

3.3. Gall induction on potted plants

After exposure to *T. acaciaelongifoliae,* galls only developed on potted *A. longifolia*. Three potted *A. longifolia* (*i.e.,* 50%) developed galls in low numbers. One plant had three galls which were 2, 4 and 6 mm in diameter, and the other two plants had one gall each, which were 7 and 9 mm in diameter. No galls developed on either of the other two species. Although the plants were healthy when presented to the wasps, some perished during the subsequent monitoring period. Nevertheless, galls were clearly visible within 2 months of exposure to the wasps and all of the plants survived for that length of time.

3.4. Surveys in South Africa and Australia

In areas where *T. acaciaelongifoliae* has open access to the environment, field surveys revealed that only *A. longifolia* had galls of *T. acaciaelongifoliae* developing on its branches and that galls were more abundant in South Africa, where the wasp is introduced, than in Australia, the native home of the wasps (Fig. 5).

4. Discussion

Even though international regulations are based on risk analysis schemes (EPPO, 2009), when deciding whether to use biological control as part of a management strategy against invasive alien plants, the risks of releasing agents must be weighed against the potential costs and benefits, including, critically in this case, whether or not suppression of *A. longifolia* in Portugal would be possible and affordable *without* the intervention of biological control. The impacts of *A. longifolia* invasions on biodiversity and conservation in Portugal are well documented (Marchante et al., 2003, 2004, 2008a,b, 2009). In addition, management interventions practiced thus far fail in the long term because the weed resurges from accumulated seed banks (Marchante et al., 2010a) and financial constrains frequently prevent follow up control. Biological control is frequently considered as the most cost effective and environmentally-sound form of weed control (Holden et al., 1992). Based on precedents in South Africa (Dennill and Donnelly, 1991; Dennill et al., 1999), it is highly likely that, were it to be released in Portugal, *T. acaciaelongifoliae* would significantly reduce the invasiveness of *A. longifolia*.

The observations of *T. acaciaelongifoliae* laying eggs on buds of *V. vinifera* and *C. striatus* in Petri dishes are in every likelihood laboratory artifacts, induced by the confined conditions and by the lack of suitable host plant material being available to the females. Confinement in cages is well known to disrupt normal behavioral (including olfactory and gustatory) responses of herbivore insects and induces them to develop on a much wider range of plants (termed the physiological host range) than they would do naturally (Heard, 2000; Marohasy, 1998; Van Klinken, 2000; Withers et al., 2000; Sheppard et al., 2005). Such a situation arose during the early stages of the only other biological control program against an invasive plant in Europe. In that case, a psyllid, *Aphalara itadori* Shinji, was being considered as a possible agent for biological control of *Fallopia japonica* (Houtt) Ronse Decraene in the UK (Shaw, 2009). Despite the ambiguous results of laboratory tests, the psyllid was approved for release early in 2010 (Shaw, 2009). The aberrant oviposition behavior of *T. acaciaelongifoliae* in this study needs to be weighed against all the other available evidence about the specificity of the wasp (*i.e.,* gall formation and field observations in South Africa and Australia). Doing so dispels any doubt that the insect is highly host specific and therefore poses no threat to any commercial or indigenous plants in Portugal.

Besides the need for assurances that *T. acaciaelongifoliae* will be restricted to *A. longifolia*, the possible acquisition of natural enemies, unsuitable climatic conditions or translocation from the southern to the northern hemisphere may limit its effectiveness in Portugal or even prevent its establishment altogether.

In South Africa, *A. longifolia* trees are generally more heavily galled than they are in Australia, where *T. acaciaelongifoliae* suffers high levels of parasitism and has to compete with other bud-feeding insects (Neser, 1984). This discrepancy persists even though *T. acaciaelongifoliae* is attacked by several native parasitoid species in South Africa (Hill and Hulley, 1995; Manongi and Hoffmann, 1995); Seymour, 2010). There is no reason to expect that indigenous hymenopterous parasitoids will not utilize the larval and pupal stages of *T. acaciaelongifoliae* in Portugal (Noyes, 2003); E. Marchante, *unpublished data*) but, because there are no ecological analogues (i.e., gall forming insects on acacias), the impact of parasitoids is likely to be trivial (Paynter, 2010).

In South Africa, *T. acaciaelongifoliae* is reported to be most effective in warm temperate areas. In terms of the Köppen-Geiger climate classification system (Kottek et al., 2006), these areas are grouped as Csb (dry summers) and Cfb (humid summers), with a threshold temperature of > 10ºC for at least 4 months of the year and a mean temperature for the hottest month of ≥ 22º C; *i.e.*, areas that are climatically similar to areas where *T. acaciaelongifoliae* was originally collected in Australia (Cfb) (Dennill, 1987). Most of the Portuguese coastal region (except for the southern extremity of the country) and the interior in the north of Portugal, extending into northwestern Spain are classified as Csb (Kottek et al., 2006). In Portugal, *A. longifolia* is invasive mainly in the coastal regions, extending to Galicia, Spain, (Elorza et al., 2004), where climatic conditions would be favorable for the development and survival of *T. acaciaelongifoliae.* Thus there should be no concerns that climatic-mismatching would dampen the performance of the wasp, were it to be released in Portugal. *Acacia longifolia* is considered naturalized in France and Italy, though it is not invasive in either of these countries (Celesti-Grapow et al., 2009; Elorza et al., 2004). Nevertheless, there are other regions in Europe which have Csb or Cfb climates (Kottek et al., 2006), so the potential for the wasp to spread across political boarders will need to be addressed in the Pest Risk Analysis that will be submitted to authorities to consider the release of the gall wasp, and other European countries will need to be kept informed of developments in Portugal.

In moving *T. acaciaelongifoliae* from South Africa to Portugal, consideration needs to be given to the asynchronous phenology of the host plant in the southern and northern hemispheres. Female wasps moved from South Africa in October/November when the adults are most abundant will be faced with host plants in late-autumn stages in Portugal when most of their buds will be too large (>1.5 mm in length) for egg deposition. At that time of year there would be some smaller buds on the plants which should enable the wasps to establish founder populations. Alternatively, lower numbers of *T. acaciaelongifoliae* females could be collected in South Africa earlier in the year (N. Dorchin, pers. comm.) and shipped to Portugal when the *A. longifolia* plants would be in a more suitable phenological stage. Either way, provided enough wasps are released over a sufficiently long period of time, some females should oviposit successfully and produce founding and then burgeoning populations of adults synchronized with the phenology of *A. longifolia* in Portugal.

All indications are that there are no substantive reasons not to release *T. acaciaelongifoliae* in Portugal and thereby alleviate the overwhelming negative impacts of *A. longifolia.* The extremely slight risk that the wasps might lay some eggs on plants other than *A. longifolia*, and the minimal consequences thereof, are more than offset by the substantial benefits that will accrue if the project succeeds. Biological control is the only way to prevent an escalation in levels of irreversible damage that *A. longifolia* will inevitably inflict on the ecology and biodiversity of whole communities of native organisms in Portugal, and further afield in Europe.

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Table 1. List of plant species tested in non-choice tests with *Trichilogaster acaciaelongifoliae*, including selection criteria for each species. (n = native species; e = exotic species).

Family			Non-target species	criteria
Anacardiaceae	1	n	*Pistacia lentiscus* L.	
Caprifoliaceae	2	n	*Viburnum tinnus* L.	
Cistaceae	3	n	*Cistus psilosepalus* Sweet	
Empetraceae	4	n	*Corema album* (L.) D.Don	
Ericaceae	5	n	*Arbutus unedo* L.	
	6	n	*Erica scoparia* L.	
Fabaceae	7	e	subfam. *Caesalpinioideae - Ceratonia siliqua* L.	
(=Leguminosae)	8	n	subfam. *Faboideae - Cytisus striatus* (Hill.) Rothm.	
	9	n	subfam. *Faboideae - Genista falcata* Brot.	
	10	n	subfam. *Faboideae – Medicago marina* L.	
	11	e	subfam. *Faboideae - Phaseolus vulgaris* L.	
	12	e	subfam. *Faboideae - Pisum sativum* L.	
	13	n	*subfam. Faboideae - Stauracanthus genistoides* (Brot.) Samp. subsp. g*enistoides*	
	14	n	subfam. *Faboideae - Ulex parviflorus* L.	
	15	e	subfam. *Faboideae - Vicia faba* L.	
	16	e	subfam. *Mimosoideae - Acacia melanoxylon* R. Br.	
Fagaceae	17	n	*Quercus faginea* Lam.	
	18	n	*Quercus lusitanica* Lam.	
	19	n	*Quercus pyrenaica* Willd.	
	20	n	*Quercus robur* L.	
	21	n	*Quercus rotundifolia* Lam.	
	22	n	*Quercus suber* L.	
	23	n	*Quercus x coutinhoi* Samp.	
Lamiaceae	24	n	*Lavandula luisieri* (Rozeira) Rivas-Martinez	
Lauraceae	25	n	*Laurus nobilis* L.	
Myricaceae	26	n*	*Myrica faya* Aiton	
Myrtaceae	27	e	*Eucalyptus globulus* Labill.	
Oleaceae	28	n	*Phillyrea angustifolia* L.	
Pinaceae	29	n*	*Pinus pinaster* Aiton	
	30	e	*Pseudotsuga menziesii* (Mirbel) Franco	
Polygalaceae	31	n	*Polygala vulgaris* L.	
Rhamnaceae	32	n	*Rhamnus alaternus* L.	
Rosaceae	33	e	*Pyrus communis* L.	
	34	e	*Prunus persica* (L.) Batsch.	
	35	n	*Prunus lusitanica* L.	
	36	e	*Malus domestica* Borkh.	
Rutaceae	37	e	*Citrus sinensis* (L) Osbeck	
Salicaceae	38	n	*Salix atrocinerea* Brot.	
Ulmaceae	39	n	*Ulmus procera* Salisb.	
Vitaceae	40	e	*Vitis vinifera* L.	

species phylogentically related (centrifugal phylogenetic method)
species with some morphological (buds, *i.e.* size, absence of indument,...) similarities
species with ecological/distribution overlap
economic plant species
species with conservation value

* Considered to be exotic by some authors

Note: Where more than one criteria was used for selection of a particular species, the relative importance of each of the criteria is indicated by the width of the blocks

Table 2. Time, mean (SE) (sec), that *Trichilogaster acaciaelongifoliae* spent on each plant species exploring the buds or oviposition*. Only species where the wasps came into contact with the buds are included

Species	stationary on bud (SE)	active on buds (SE)	probing (SE)
Acacia longifolia	**115.4** (68.0)	**36.4** (36.4)	**64.3** (47.4)
Acacia melanoxylon	**231.1** (119.8)	**105.1** (90.8)	**10.9** (7.4)
Corema album	**15.5** (15.5)	--	--
Cytisus striatus	**270.6** (128.3)	--	**96.7** (96.7)
Erica scoparia	--	--	**42.8** (42.8)
Genista falcata	--	**4.7** (4.7)	--
Laurus nobilis	--	**46.2** (46.2)	**57.4** (57.4)
Malus domestica	**99.0** (99.0)	--	--
Medicago marina	--	**2.1** (2.1)	--
Myrica faya	--	**21.3** (21.3)	--
Phillyrea angustifolia	--	**84.2** (55.3)	--
Pinus pinaster	**2.3** (2.3)	**3.8** (3.8)	--
Pyrus communis	--	**9.0** (9.0)	--
Quercus faginea	**6.6** (6.6)	**6.3** (6.3)	**3.2** (3.2)
Rhamnus alaternus	--	**12.9** (12.9)	--
Ulex parviflorus	**74.3** (54.1)	--	--
Ulmus procera	**47.4** (31.5)	--	--
Vitis vinifera	--	**17.5** (17.5)	**2.5** (2.5)
One-Way ANOVA:	$F_{8, 91} = 1.434$, $p = 0.193$	$F_{11, 110} = 1.011$, $p = 0.422$	$F_{6, 72} = 0.105$, $p = 0.996$

* **Active on buds,** wasps walking on the bud with their antennae not in contact with the bud. **Stationary on buds,** wasps stationary on the buds. **Probing**, wasps with the ovipositor inserted into the bud, but not always associated with egg deposition which was confirmed by dissection of buds.

"—" denotes behavioral element was not registered on the species.

Figures captions

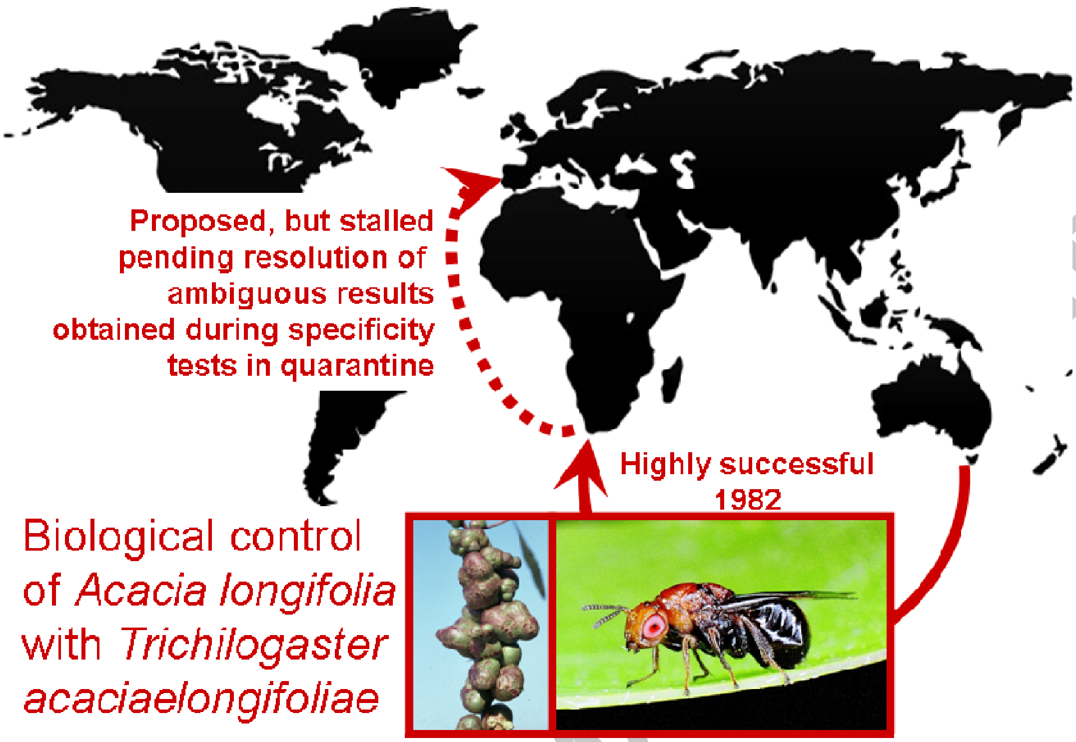
Fig. 1. Percentage of branches where eggs of *Trichilogaster acaciaelongifoliae* were detected amongst all of the plant species tested. The species are ordered (from top to the base) according to phylogenetic closeness to *Acacia longifolia*.

Fig. 2. Number of eggs (mean +SE per branch) laid on *Acacia longifolia* and the three non-target where eggs were detected (Tukey test, $P < 0.05$).

Fig. 3. Percentage of different-sized buds that had eggs of *Trichilogaster acaciaelongifoliae* on four different plant species ($n = 9$ females exposed on each plant species).

Fig. 4. Number (mean + SE) of eggs of *Trichilogaster acaciaelongifoliae* in different sized buds of four plant species. Columns with the same letters are not significantly different from each other (Tukey test, $P < 0.05$).

Fig. 5. The abundance of galls of *Trichilogaster acaciaelongifoliae,* on the terminal 70 cm of branches, on five plant species including the target, *Acacia longifolia,* in South Africa (SA) (Western Cape) and Australia (A) (New South Wales). The non-target species included two species (*Vitis vinifera* and *Acacia melanoxylon*) where the wasps laid eggs during no-choice tests, and two species closely related to *Cytisus striatus*. Non-target species were sampled in SA and A, except for *Spartium junceum*.


Proposed, but stalled
pending resolution of
ambiguous results
obtained during specificity
tests in quarantine
Highly successful
1982
Biological control
of *Acacia longifolia*
with *Trichilogaster*
acaciaelongifoliae

Research highlights:

- Eggs laid by *T. acaciaelongifoliae* on ‘non-hosts’ in quarantine
- Tests on potted plants and field surveys abroad confirmed findings were false positives
- The extremely slight risk that the wasps might lay some eggs on ‘non-hosts’, and the minimal consequences thereof, are more than offset by the substantial benefits that will accrue if the project succeeds

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Figure 1

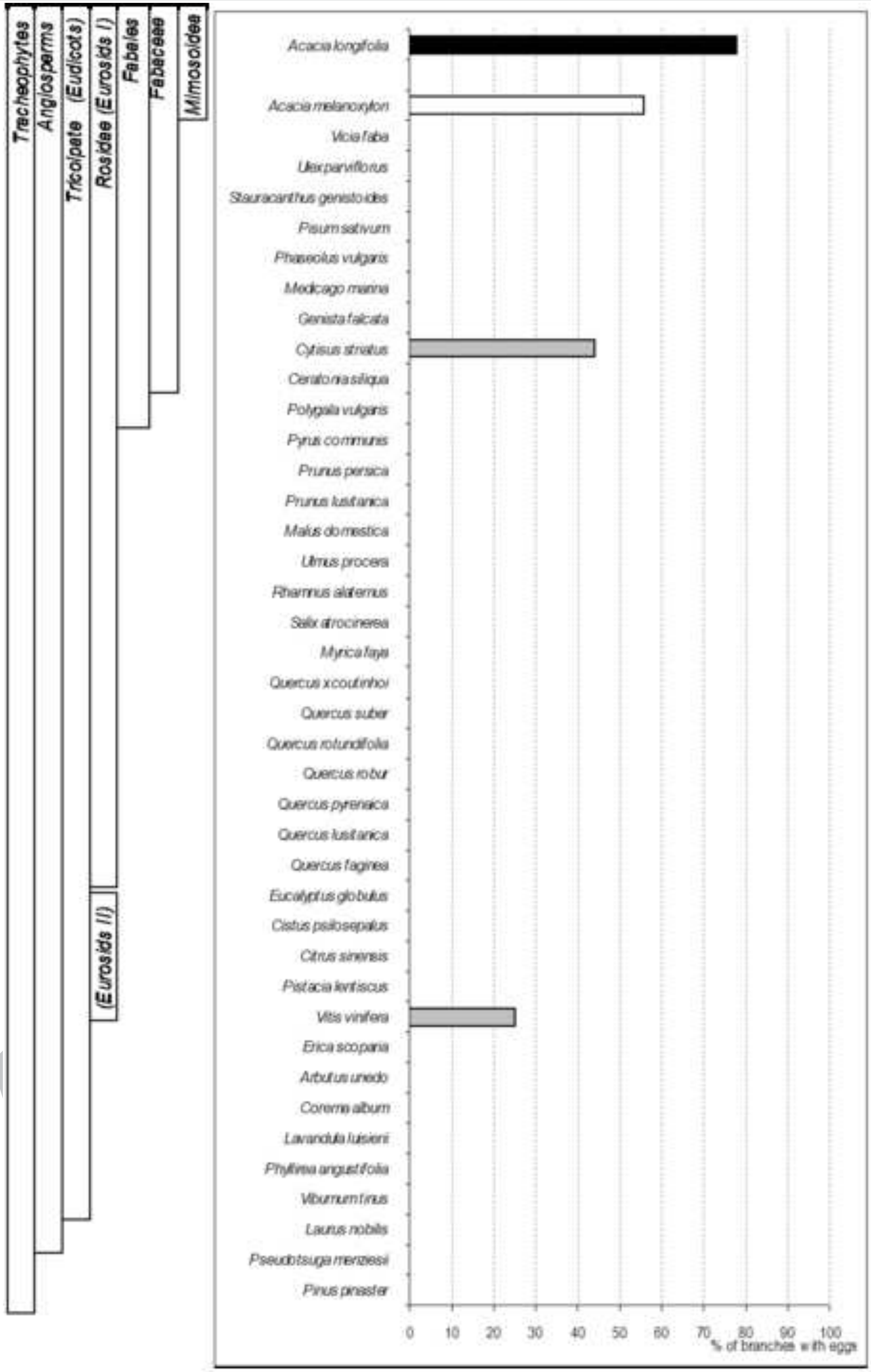

Tracheophytes
Angiosperms
Tricolpate (Eudicots)
Rosidae (Eurosids I)
Fabales
Fabaceae
Mimosoidae
(Eurosids II)
Acacia longifolia
Acacia melanoxylon
Vicia faba
Ulex parviflorus
Stauracanthus genistoides
Pisum sativum
Phaseolus vulgaris
Medicago marina
Genista falcata
Cytisus striatus
Ceratonia siliqua
Polygala vulgaris
Pyrus communis
Prunus persica
Prunus lusitanica
Malus domestica
Ulmus procera
Rhamnus alaternus
Salix atrocinerea
Myrica faya
Quercus xcoutinhoi
Quercus suber
Quercus rotundifolia
Quercus robur
Quercus pyrenaica
Quercus lusitanica
Quercus faginea
Eucalyptus globulus
Cistus psilosepalus
Citrus sinensis
Pistacia lentiscus
Vitis vinifera
Erica scoparia
Arbutus unedo
Corema album
Lavandula luisieri
Phyllirea angustifolia
Viburnum tinus
Laurus nobilis
Pseudotsuga menziesii
Pinus pinaster
0 10 20 30 40 50 60 70 80 90 100
% of branches with eggs

Figure 2

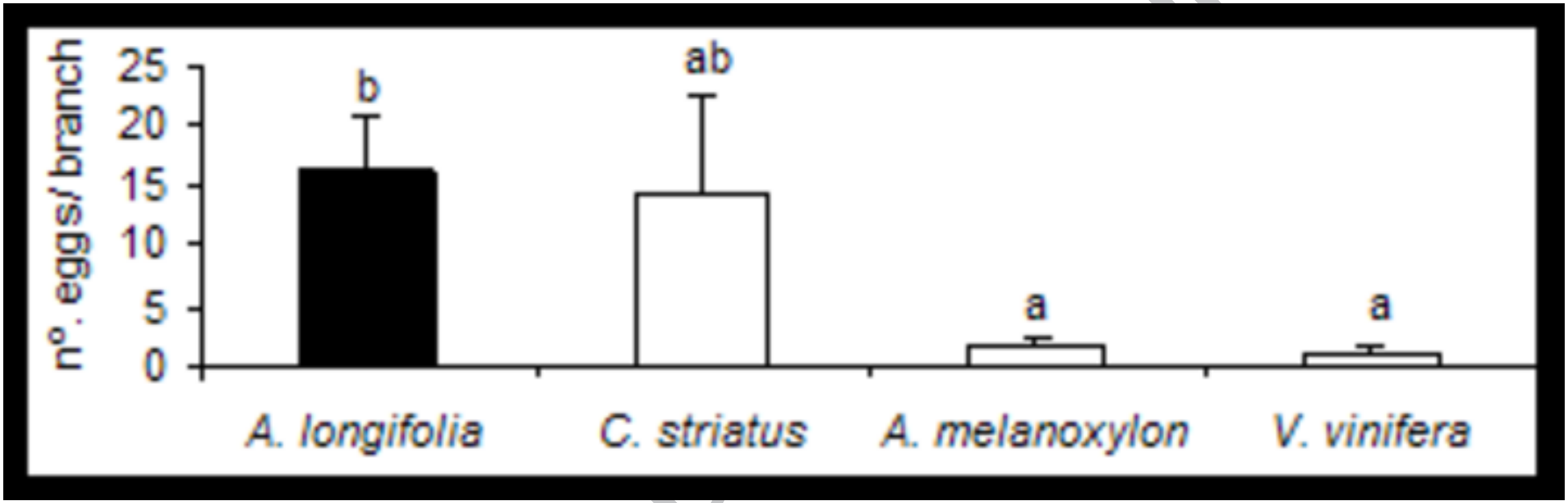

n°. eggs/ branch
25
20
15
10
5
0
b
ab
a
a
A. longifolia
C. striatus
A. melanoxylon
V. vinifera

Figure 3

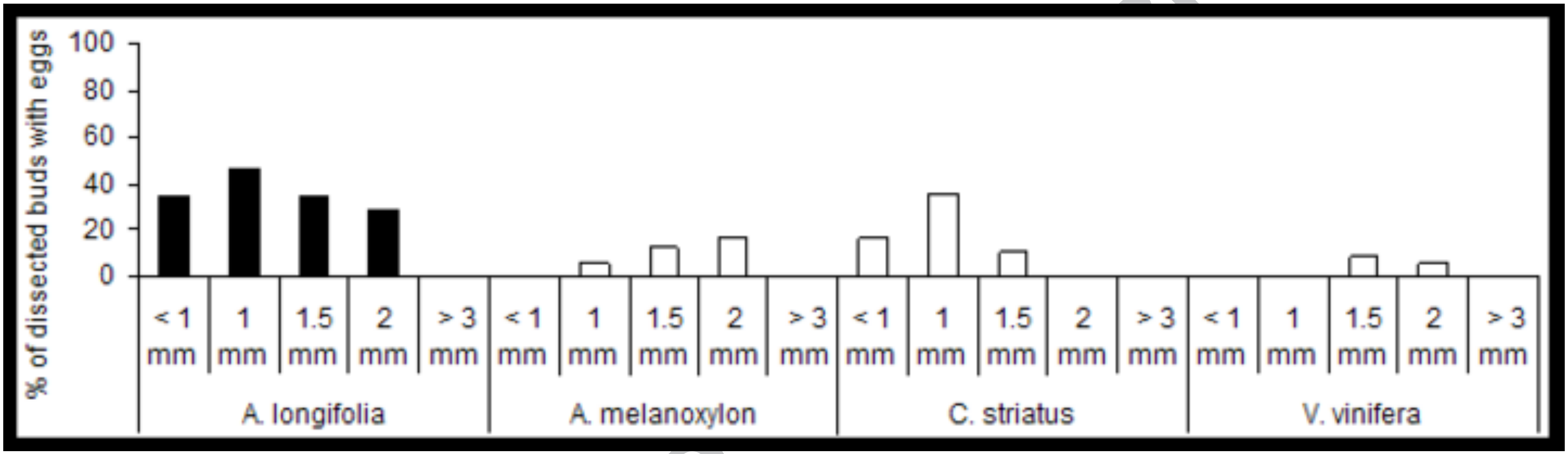

% of dissected buds with eggs
100
80
60
40
20
0
< 1 mm
1 mm
1.5 mm
2 mm
> 3 mm
A. longifolia
A. melanoxylon
C. striatus
V. vinifera

Figure 4

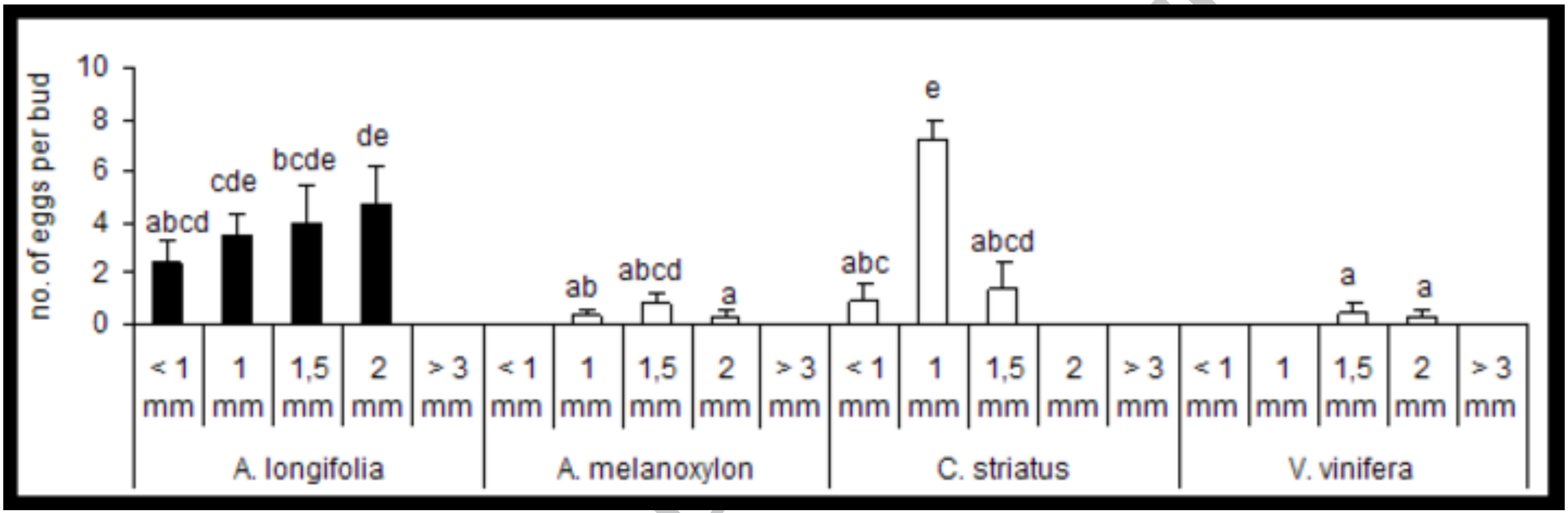
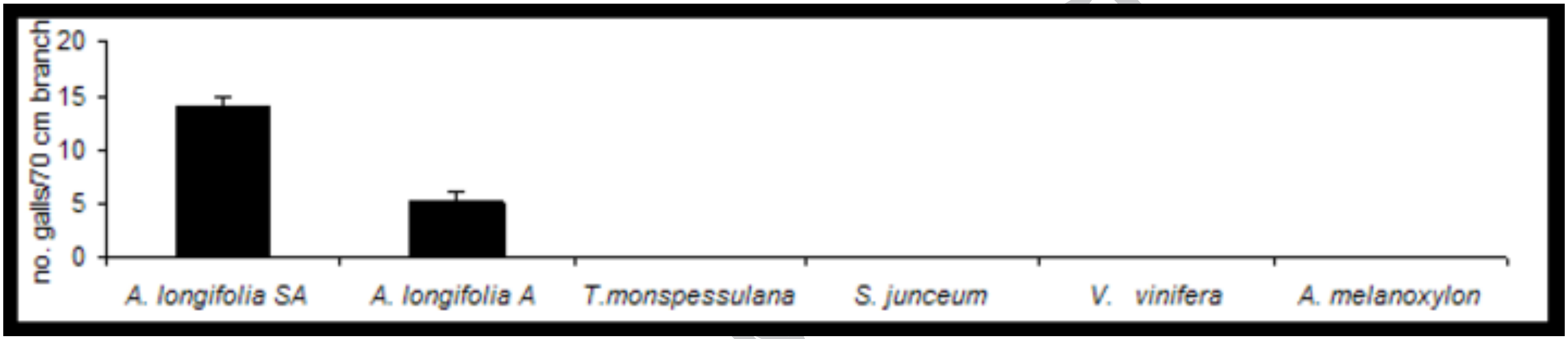

no. of eggs per bud
10
8
6
4
2
0
abcd
cde
bcde
de
ab
abcd
a
abc
e
abcd
a
a
< 1 mm
1 mm
1,5 mm
2 mm
> 3 mm
A. longifolia
A. melanoxylon
C. striatus
V. vinifera

Figure 5



no. galls/70 cm branch
20
15
10
5
0
A. longifolia SA
A. longifolia A
T.monspessulana
S. junceum
V. vinifera
A. melanoxylon