Tittle

Establishment, spread and early impacts of the first biocontrol agent against an invasive plant in continental Europe

Authors

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Abstract

Classical biocontrol is key for the successful management of invasive alien plants; yet, it is still relatively new in Europe. Although post-release monitoring is essential to evaluate the effectiveness of a biological control agent, it is often neglected. This study reports the detailed post-release monitoring of the first biocontrol agent intentionally introduced against an invasive plant in continental Europe. The Australian bud-galling wasp *Trichilogaster acaciaelongifoliae* (Frogatt) is used to control the invasive *Acacia longifolia* (Andr.) Willd., with a long history of success in South Africa. This biocontrol agent was first released in Europe in 2015 at several sites along the Portuguese coast. We monitored the establishment, spread and early impacts of *T. acaciaelongifoliae* on target-plants in Portugal, across 61 sites, from 2015 to 2020. Initial release of adults emerging from galls imported from South Africa and the subsequent releases from galls established in Portugal (2018 onwards) was compared, assessing the implications of the hemisphere shift. The impacts on the reproductive output and vegetative growth of *A. longifolia* were evaluated in more detail at three sites. From 2015 to 2019, 3,567 *T. acaciaelongifoliae* were released at 61 sites, with establishment confirmed at 36 sites by 2020. The transfer of the wasp from the southern hemisphere limited its initial establishment, but increased rates of establishment followed with synchronization of its life cycle with northern hemisphere conditions. Therefore, after an initial moderate establishment, *T. acaciaelongifoliae* adapted to the northern hemisphere conditions and experiences an exponential growth (from 66 galls by 2016, to 24,000 galls by 2018). Galled *A. longifolia* branches produced significantly fewer pods (-84.1%), seeds (-95.2%) and secondary branches (-33.3%) and had fewer phyllodes but increased growth of the main branch compared to ungalled branches. *Trichilogaster acaciaelongifoliae* successfully established in the northern hemisphere, despite the initial phenological mismatch and adverse weather conditions. To achieve this, it had to establish and synchronize its life cycle with the phenology of its host-plant, after which it developed exponentially and began to show significant impacts on the reproductive output of the target plant.

Keywords

bud-galling wasp, hemisphere shift, invasive plant management, phenological mismatch, post-release monitoring, Sydney golden wattle

Research highlights

1. A biocontrol agent for the invasive *Acacia longifolia* has established in Portugal
2. The asynchrony between the target-plant and the biocontrol agent is being overcome
3. A southern biocontrol agent is successfully spreading in the northern hemisphere
4. The biocontrol agent is reducing the growth and seed production of the target-plant
5. Post-release monitoring of biocontrol agents, is crucial for biocontrol safety

1. Introduction

With over 1,500 species introduced and spread around the globe (Turbelin et al., 2017), invasive alien species are one of major threat to biodiversity (IPBES, 2019). After overcoming biogeographical barriers with human assistance, they can establish self-perpetuating populations in the introduced range, influencing the dynamics of the ecosystems they invade (Richardson et al., 2000), often with negative consequences (Kumar Rai and Singh, 2020; Rumlerová et al., 2016). *Acacia longifolia* (Andr.) Willd. (Sydney golden wattle; Fabaceae: Mimosoideae) is one of such invasive species. It is a shrub/small tree native to south-eastern Australia, that was mainly introduced in the last century into several countries to stabilize sand dunes and for ornamental purposes (Kull et al., 2011). Nowadays, it is a widespread invasive species in many countries, including Argentina, Brazil, New Zealand, Spain, South Africa, and Portugal where it dominates coastal, riverine and montane systems (CABI, 2020). Its fast growth and competitive ability (Werner et al., 2010), the pyrophytic behaviour, and the abundant production of seeds (up to 11,500 seeds/m^2/year) (Gibson et al., 2011), which can be viable in soil for a long time (Marchante et al., 2010), contribute to *A. longifolia* invasive capacity. It is broadly recognized that the dense and monospecific *A. longifolia* stands, reduce biodiversity and disrupt the nutrient and water cycles and fire regimes (Le Maitre et al., 2011; López-Núñez et al., 2017; Marchante et al., 2015).

Classical biocontrol of invasive plants takes advantage of host-specific natural enemies selected from the native range of the invasive host-plant, and promotes their subsequent release in areas where the target-species is invasive, reducing its biomass, flowering or seed production, hampering the spread of the target-population (Stiling and Cornelissen, 2005). If effectively implemented, classical biocontrol, may be a more sustainable and environmental-friendly alternative to mechanical and chemical control methods (Shaw et al., 2018; Simberloff, 2014; van Wilgen et al., 2020), with a lower long-term cost/benefit ratio and greater likelihood of preventing re-invasion (Van Driesche et al., 2010). A recent review has shown that classical biocontrol successfully reduced the negative impacts of invasive plants, with ca. 50% of releases resulting in some level of control and ca. 25% resulting in heavy control of the invasive plant species (Hinz et al., 2020). However, despite the fact that classical biocontrol has been used for over a century in many parts of the world, its use in Europe is still recent, and often viewed with scepticism (Shaw et al., 2018). *Trichilogaster acaciaelongifoliae* (Froggatt) (Hymenoptera: Pteromalidae) is a univoltine (i.e. one generation per year) and parthenogenic bud-galling wasp, native to south-eastern Australia and specific to *A. longifolia s.l.* (Noble, 1940). In the southern hemisphere, the wasps emerge from the galls in late summer, with peak emergence in November. Adult wasps live for 1–3 (rarely up to 7) days, and females oviposit on average 300-400 eggs, preferably in small floral buds (< 2 mm), and less frequently in vegetative buds, inducing the formation of a gall, and preventing seed formation and vegetative growth (Noble, 1940). The wasp was first released in South Africa in 1981, where it has proved a successful biocontrol agent, reducing *A. longifolia* reproductive output (up to 95% less seed) and vegetative growth (decrease of 30%) (Dennill, 1988, 1985). After the success achieved in South Africa, the use of *T. acaciaelongifoliae* was considered in Portugal, and after host-specificity testing and a lengthy process for obtaining release approval, the wasp was imported from South Africa and released along the Portuguese coast in 2015 (Marchante, Freitas, & Hoffmann, 2011; Shaw, Schaffner, & Marchante, 2016). The first galls were reported in 2016 at four sites (Marchante et al., 2017), but at that time it was too early to evaluate its performance, establishment and early effects on *A. longifolia*. We aim to do that in the present study. *Trichilogaster acaciaelongifoliae* was only the third intentionally introduced biocontrol agent for an invasive plant in Europe, and the first on the mainland following two biocontrol releases in the UK (EPPO, 2010; Marchante et al., 2017; Tanner et al., 2015), stressing the need and novelty of the present study.

The establishment of a biocontrol agent is influenced by many factors such as the number of agents released and the timing of such releases, the weather conditions (Crawley, 1989), and the phenological synchrony between the phenology of the host-plant and the biocontrol agent (Gupta et al., 2016). Although Schwarzländer, Hinz, Winston, & Day (2018) reported a generally high establishment rate (70.9%) of 468 intentionally-introduced agents used against invasive plant species, the implications of translocation across hemispheres were still not analysed (Winston et al., 2014). This was however a concern regarding the introduction of *T. acaciaelongifoliae* into Portugal from South Africa. During release campaigns of wasps imported from South Africa (November – early January), the number of *A. longifolia* plants with suitably sized buds for *T. acaciaelongifoliae* to oviposit in Portugal was low as the majority of suitable buds (Dennill, 1987) develop earlier in the year, between May and June (Morais & Freitas, 2015). This asynchrony in the host phenology was an issue that could impede the wasp establishment in the northern hemisphere (Marchante et al., 2017). It was thus essential to monitor the initial establishment and spread of *T. acaciaelongifoliae* and to assess if it would be able to synchronize its life cycle with the phenology of *A. longifolia* in the new hemisphere. The post-release monitoring of biocontrol programs is crucial to test agent effectiveness, detect potential non-target effects, allow a truly adaptive management, and increase the support for safe biocontrol (McFadyen, 1998; Morin et al., 2009; Schaffner et al., 2020). However, the post-release monitoring assessments are often neglected and fragmented and in Europe are still in their infancy (Clewley, 2014; Ellison et al., 2020; Schaffner et al., 2020). To the best of our knowledge, this is only the third post-release assessment of a biocontrol agent intentionally introduced in Europe (Clewley, 2014; Ellison et al., 2020), offering excellent opportunities to advance theoretical and applied ecological knowledge (Schaffner et al., 2020; Shaw et al., 2018). In this context, our aims were: 1) monitor the establishment and early spread of *T. acaciaelongifoliae* in Portugal; 2) analyse the implications of hemisphere shift in the establishment of the wasp by comparing the establishment of imported and Portuguese populations; and 3) assess the initial impacts of *T. acaciaelongifoliae* on the vegetative and reproductive output of the target-plant, *A. longifolia*.

2. Materials and Methods

2.1. Study site

This study was carried out along the 700 km of the Portuguese Atlantic coast. This area is characterised by secondary sand dunes where a rich native vegetation including, *Stauracanthus genistoides* (Brot.) Samp. and *Corema album* (L.) D.Don has been largely replaced by *A. longifolia* and other invasive plants species such as *Carpobrotus edulis* (L.) N.E.Br.. The climate is mostly Mediterranean with warm-summers (Csb), but with hotter-summers in the southern-most region (Csa) (Kottek et al., 2006), a climate very similar to the invasive range of *A. longifolia* in South Africa.

In order to increase the establishment probability and promote a continuous distribution of *T. acaciaelongifoliae* across the country, releases were performed in 61 sites, mostly along the coast, where no previously establishment has been recorded (Fig. 1 and Supplementary Materials: Table S1). Between November and early January of 2015, 2016 and 2017, 2,073 mature *T. acaciaelongifoliae* galls were imported from field populations at ARC-PHP (Agriculture Research Council-Plant Health and Protection) Vredenburg Campus, Stellenbosch (South Africa) (Lat -33.9497167, Long 18.8360446) and kept in quarantine conditions in Portugal upon arrival. Healthy, females, *T. acaciaelongifoliae* were released into pre-selected sites within 36 hours of emergence (to minimize mortality risk). In June 2018, 1,091 mature galls were collected from a newly-established population in São Pedro de Moel (Portugal). These were maintained under similar laboratory conditions as the imported galls and healthy newly-emerged females were released as described above. In 2019, 73 additional galls from established Portuguese populations were released into two additional sites. Between 2015 and 2019, 3,567 wasps were released into 61 sites (Fig. 1 and Table 1).

2.2. Release method for *Trichilogaster acaciaelongifoliae*

Four of the 61 release sites (São Jacinto dunes, Quiaios, Coimbra and São Pedro de Moel; see Table 1) were selected for regular monitoring and variable numbers of *T. acaciaelongifoliae* females were released onto at least 25 healthy *A. longifolia* trees at each of these sites. Releases at the remaining 57 sites depended on the availability of *A. longifolia* trees and newly-emerged females (Table 1). Trees on which releases were made were at least 20 m apart and, on each tree, three branches exhibiting immature healthy buds (< 2 mm) were selected, marked, and a single wasp was gently placed on one phyllode of each of the three branches, close to a suitable bud. Branches on trees at the four regularly monitored sites were geolocated with sub-metric accuracy GPS (Trimble GeoExplorer XT 6000) to record the initial dispersal point. Wasps from the imported galls all emerged during the winter in the northern hemisphere and were released between November 2015, 2016 and 2017 and early January of the following year. Conversely, the wasps emerging from galls collected in Portugal emerged during the summer and were released in June - July of 2018 and 2019.

2.3. Establishment and monitoring of *Trichilogaster acaciaelongifoliae*

Establishment success (i.e., galls presence) was assessed on all 61 release sites, and four of them (see above), were selected for a more detailed monitoring. To evaluate the presence of *T. acaciaelongifoliae*, the four sites were visited monthly and all release trees inspected between March and July, which coincided with the peak period of floral and vegetative development. In addition, all release trees were re-visited once in autumn and again in winter to deal with the associated uncertainties about the synchrony of the life cycle of *T. acaciaelongifoliae* with its host in the northern hemisphere, making it difficult to readily predict the timing of gall development. After 2017, *T. acaciaelongifoliae* populations appeared to have become synchronised with *A. longifolia* in Portugal, and further monitoring was limited between late spring and early summer. On each visit, marked branches were inspected and the following information was recorded: i) Number of galls per branch; ii) Number of chambers per gall (externally accessed); and iii) the GPS coordinate of each gall (or cluster of galls when these were physically attached). From 2016 to 2017 new galls were actively searched for in the vicinity of the release trees. This was done by heading approximately 20 m from each release tree in the direction of the four cardinal points until no *T. acaciaelongifoliae* galls were observed, and all galls were counted. By 2018, the exponential growth in the number of *T. acaciaelongifoliae* galls at the four regularly monitored sites made it virtually impossible to maintain an absolute count as before. Consequently, for each *A. longifolia* with galls, a subsample of galled branches was randomly selected and the total number of galls on the tree estimated based on the total number of branches. By 2019, high gall loads and widespread distribution of the wasp within sites made it impossible to detect all galled branches. Therefore, gall density was estimated using two complementary methods that provide an effective and replicable monitoring strategy for the long-term: 1) In Quiaos, São Pedro de Moel and Coimbra, gall density (galls/m^2) was estimated based on the total number of galls counted along three transects of 20 x 2 x 1 m (length x width x height) per site; 2) In São Jacinto dunes, gall density was estimated from an extrapolation via an active search for galls on a grid of 4.3 x 1.5 km (cell size 100 x 200 m; 3,423 cells monitored; total size 686 ha) in late July. Cells without suitable habitats for *T. acaciaelongifoliae* were excluded from the survey. The number of observed *T. acaciaelongifoliae* galls were recorded in the centroid of each cell by an observer scanning the environment within a 50 m radius with binoculars for 3 minutes. Between 2015 and 2017, at the four regular sampling sites the maximum annual dispersal distance of the wasp from the release trees at the four regular sampling sites, was estimated using ArcGIS v10.6.1.

2.4. Impact of *Trichilogaster acaciaelongifoliae* on *Acacia longifolia*

The impact of *T. acaciaelongifoliae* on vegetative and reproductive growth of *A. longifolia* was assessed at three of the monitoring sites (São Jacinto dunes, São Pedro de Moel, and Quiaios). The fourth site, at Coimbra, was excluded from the study due to forestry activities during 2019. At both São Jacinto dunes and São Pedro de Moel, 48 trees were used, however, only 25 trees were used at Quiaios due to low *T. acaciaelongifoliae* galling. A total of 59 galled trees and 62 ungalled trees were assessed at these sites between 2018 and 2020. On each of the galled trees, five galled branches and five ungalled branches were randomly selected; on ungalled trees, only five branches were randomly selected (Fig. 2). All selected branches were marked, geolocated and measured: i) Length of the branch from the tip to the very first branching, ii) Number of secondary branches, iii) Number of phyllodes, iv) Number of pods and v) Number of seeds. A total of 390 branches were marked in 2018 and re-measured in 2019, and an additional 510 branches (on new trees) were marked in 2019 and re-measured in 2020.

2.5. Statistical analysis

The population growth and spread of *T. acaciaelongifoliae* from 2016 to 2018 was analysed with two Generalized Linear Mixed Models (GLMM) including respectively the number of galls and the maximum dispersal distance (log transformed) and site included as a random factor. The impacts of *T. acaciaelongifoliae* on the vegetative and reproductive growth of *A. longifolia* was also analysed using GLMMs for each measured variable and comparisons drawn between the change in proportions in the vegetative and reproductive outputs between consecutive years $(\mathrm{t1}/((\mathrm{t0}+1)\,) \times 100)$ on galled and ungalled branches, and in galled and ungalled trees (transformed as $\mathrm{sign}(x) \times \log(|x|+1)$). Each tree was modelled as a nested random factor of the year in the analysis to control the influence of inherited ontogeny on branch development. Differences between years and treatments (*i.e.*, ungalled branches on ungalled trees, galled and ungalled branches in galled trees) were further explored with a Tukey post-hoc test whenever significant impacts were detected in the GLMMs. The net impacts on *A. longifolia* were estimated by using the differential proportion between the pooled average of galled and ungalled branches in galled trees and, the ungalled branches in ungalled trees for each vegetative and reproductive variable.

All statistical analyses were performed using lme4 (Bates et al., 2015) and multcomp (Hothorn et al., 2008) in R v.3.6.1 (R Core Team, 2019).

3. Results

3.1. Establishment and spread of *Trichilogaster acaciaelongifoliae*

By 2020, five years after initiating releases of *T. acaciaelongifoliae* in Portugal, establishment had been confirmed at 36 of the 61 release sites along ca. 700 km of the Portuguese coast (Fig. 1 and Supplementary Materials: Table S1). Establishment success varied over the years, ranging from 0% in 2018 (from South African wasps released at 16 sites in 2017) to 84.8% in 2019 and 100% in 2020 (Portuguese wasps released in 33 sites in 2018 and 2 sites in 2019, respectively; Fig. 3). The number of galls increased dramatically over the years, with some 24,793 galls being accounted for in 2018, which was the last year when it was possible to estimate absolute gall numbers (Table 2). Between 2016 and 2018, the number of observed galls (with a number of chambers per gall of 2.19±0.02 Mean±SE, ranged between 1 and 12) significantly increased ($F_2 = 36.113$, $P < 0.001$) every year in relation to the previous year, especially from 2017 to 2018 (from 331±136 to 6,167±3,593 Mean±SE, respectively) (Fig. 4b). In 2019, gall numbers continued to increase, reflected in a maximum of 298 galls/m^2 at Coimbra (Table 1). However, as the monitoring method had to be adapted to manage the increased gall loads, it was not possible to statistically compare these results. The dispersal distance also increased significantly ($F_2 = 6.324$, $P < 0.05$) from 2015 to 2018 (Fig. 4a and c), with *T. acaciaelongifoliae* galls initially being found only in close proximity to the release trees (3.31±2.56 m), but subsequently being detected at 13.17±5.13 m and 223.30±195.31 m from the release trees, one and two years after release, respectively. In 2019, some galls were observed 7 km away from the closest release tree (unpublished data).

3.2. Impacts of *Trichilogaster acaciaelongifoliae* on *Acacia longifolia*

Given the extent of invasions of *A. longifolia* in Portugal, the total number of galled trees remains relatively low. However, some positive impacts of *T. acaciaelongifoliae* are already being observed on the reproductive and vegetative growth of *A. longifolia* with just a few years of establishment. The production of pods and seeds were significantly reduced in galled trees when compared to ungalled trees (84.1% and 95.2%, ; $F_2 = 27.155$, $P < 0.001$ and $F_2 = 22.672$, $P < 0.001$, respectively), and the reduction was greater when comparing galled branches of galled trees with ungalled branches of ungalled trees.

The number of secondary branches was also significantly reduced (33.3%) ($F_2 = 7.522$, $P < 0.001$) in galled trees independently on whether these branches were galled or not. Galled trees tend to produce fewer phyllodes than ungalled trees (28.5%), which again was independent of branches having galls or not, but this trend was not significant ($F_2 = 1.265$, $P = 0.285$). Branches tend to grow more (17.5%) on galled trees compared to ungalled trees, independently of the branches having galls or not, but again this difference was not significant ($F_2 = 1.405$, $P = 0.248$) (Fig. 2 and Fig. 5).

4. Discussion

Following the first releases in 2015, *T. acaciaelongifoliae* has successfully established on *A. longifolia* along the coastal areas in Portugal. Our results show that the introduction of a biocontrol agent with an annual life cycle, from the southern into the northern hemisphere, was initially slow but successful. Results further show that the establishment success of adult wasps emerging from the first Portuguese galls was higher than that of imported galls. This increase in establishment success is largely explained by the time (season) of release, as imported wasps came from southern hemisphere summer and were released during the northern hemisphere winter, while Portuguese wasps were collected and released in late spring/summer. After introduction in Portugal, *T. acaciaelongifoliae* adapted to the local conditions and synchronized its life cycle with the host-plant phenology and the northern hemisphere seasons, facilitating further establishment. The phenological asynchrony between host-plants and their biocontrol agents has been shown to affect the success of biocontrol programs (Gupta et al., 2016; Müller et al., 1990), as the target host organs/tissues may not be available in the most appropriate development stage for the biocontrol agent (Marchante et al., 2011; Morais and Freitas, 2015). This is especially critical for highly specific agents, such as galling insects. When imported *T. acaciaelongifoliae* was released in winter (from imported South African galls), there were very few *A. longifolia* buds with a suitable size (< 2 mm) for oviposition (Marchante et al., 2011), contributing to the low success rate. Conversely, during late spring and early summer, when wasps that had developed in the wild in Portugal were released, higher numbers of suitable *A. longifolia* buds are present (Morais and Freitas, 2015). The high establishment success using wasps from populations that had established in Portugal is similar to that observed in South Africa when *T. acaciaelongifoliae* was introduced from Australia (same hemisphere) (Dennill, 1987). Furthermore, adverse climatic conditions (derived from a winter weather, such as a low temperatures, a locally high frequency of frost, a prolonged drought, etc…), to which the imported South African wasps were exposed to upon release are likely to have effected mortality of the ovipositing females, in addition to decreased mobility and the absence of necessary oviposition cues (Neser, 1984).

A comparison of release effort at the four main monitoring sites in Portugal in 2015 and initial releases in Stellenbosch, South Africa in 1981, indicate that slightly fewer wasps were released in South Africa [286 and 265 wasps, respectively; (Dennill, 1987)]. Considering that each gall has on average 2.19 chambers (each of them with one wasp), the development of second generation galls in Portugal was much lower (approx. 2,352 wasps) than the reported > 33,583 adults wasps in South Africa (Dennill, 1987), confirming that *T. acaciaelongifoliae* had to overcome some compatibility barriers (plant phenology and weather) upon its introduction in Portugal. Despite the establishment rate varies amongst localities, the number of first generation galls in South Africa (rates between 4.9 and 10.9) (Dennill, 1987) is much more comparable to that at sites where galls of Portuguese populations were released in 2018 (rates often 0.1 and 10.2) and not with those sites where the released galls were imported from South Africa (all rates < 0.4).

Although following initial release, dispersal distances of *T. acaciaelongifoliae* have increased with time in Portugal, in general, *T. acaciaelongifoliae* disperses relatively slowly, with females flying between close branches and trees, while searching for suitable buds to oviposit (Dennill, 1985). However, aided by the wind and by their excellent host-searching ability, females can cover long distances [up to 20 km (Dennill, 1987)] establishing new populations further away. Unfortunately, the detection of such long-distance dispersal events is very difficult in such small and inconspicuous animals, and therefore distances reported here should be considered as a minimum. Additionally, the increasing frequency of extreme weather and climatic events such as heat waves, together with the droughts that Portugal has been experiencing since the early twentieth century (Mora and Vieira, 2020) may affect establishment and spread of the wasp. *Trichilogaster acaciaelongifoliae* galls are sensitive to desiccation since they lack the ability to regulate evapotranspiration, negatively influencing the development of immature stages within the galls (Dennill and Gordon, 1990). Coastal dunes, the most impacted habitat by *A. longifolia* invasion (Marchante et al., 2003) and where *T. acaciaelongifoliae* releases were performed, are typically dry, with little protection from the sun, what can limit or damage *A. longifolia* buds or even impair the viability of *T. acaciaelongifoliae* eggs or larvae (Dennill and Gordon, 1990).

Although the impacts of *T. acacielongifoliae* were only measured in 2019 and 2020, a decrease in the number of pods and seeds was evident, showing that the wasp is reducing pod production (84.1%) and consequently seeds (95.2%). Results also suggest that in addition to a reduction in pod production, there was also a decline in the number of seeds per pod. The higher availability of reproductive *A. longifolia* buds for oviposition during spring could explain the greater impact of *T. acaciaelongifoliae* recorded on reproductive output [also observed in South

Africa by Dennill (1985) with a reduction of 95.5% of seeds production], rather than on vegetative growth of the plant. Additionally, galls are able to compete for nutrients with other plant organs (i.e., leaves, roots, pods) (Oliveira et al., 2016), suggesting that preference for reproductive, rather than vegetative buds for oviposition may reduce competition for resources between galls and pods and improve insect fitness (Dorchin et al., 2006). Consequently, this may also explain the differential investment of *A. longifolia* in vegetative growth after *T. acaciaelongifoliae* colonization, decreasing the growth of secondary branches (33%) and the number of phyllodes (28.5%), while increasing the length of the main branches (17.5%). Possibly, as the galls prevent the formation of secondary branches (and therefore more phyllodes), the plant might have more resources available to the growth of the main branch. This has been described for the bud-galling wasp, *Trichilogaster signiventris* (Girault) on its host-plant *Acacia pycnantha* Benth. (Hoffmann et al., 2002), although is not reported for *T. acaciaelongifoliae* in South Africa (Dennill, 1985). The tendency for an elongation of galled branches could be because the carrying capacity of *A. longifolia* has not yet been reached in Portugal, where gall densities are still relatively low compared to those measured in South Africa (Dennill, 1987, 1985). Finally, galls are a nutrient sink and a stress factor, influencing the architecture, reproduction and physiology of the host-plant (Oliveira et al., 2016), and thus ungalled branches in galled trees also show signs of stress.

4.1. Study limitations

Here we report on the scientific findings emerging from an applied conservation program, which naturally poses some limitations compared to a purely scientific experimental design. The areas invaded by *A. longifolia* are very extensive and heterogeneous. High tree density often limits the movements through the stands and management interventions (e.g., logging, forest thinning) also interfere with planned experimental design. Therefore, while the trends in establishment and dispersal of *T. acaciaelongifoliae* are obvious, gall abundance might have been underestimated (spatially and seasonally). Establishment success could also have been underestimated due to undetected galls, i.e., lack of evidence for more establishment cannot be deemed as evidence of less generalized establishment. Another limitation was that since we wanted to release and monitor *T. acaciaelongifoliae* throughout the area invaded by *A. longifolia*, given the medium to long term scope of the study, the number and extent of sites meant that there would be some level of risk/uncertainty and would also require adaptations to the field methodologies. In several instances, despite the request for managers to protect sites as far as possible, interference affected the establishment and persistence of *T. acaciaelongifoliae* populations. For example, forest fires (see Table 1), forest interventions, illegal logging, and extreme storms affected several sites. These are however common processes naturally affecting biocontrol agent establishment and population dynamics in the wild, and therefore confirming establishment and persistence under such natural setting is of utmost importance.

5. Conclusion

This work documents the enormous effort to implement biocontrol of *A. longifolia* at the national level in Portugal and demonstrates the initial success of the first intentionally released biocontrol agent in continental Europe, despite the adversities caused by the hemisphere shift. After an initially slow establishment and spread, the biocontrol wasp entered a period of accelerated population growth. Our results provide important insights on the rapid adaptation of a univoltine hymenopteran after a hemisphere translocation. Although we focus on the early impacts of the biocontrol agent, a significant impact on the reproductive potential of the target plant has already been observed, as well as a tendency for vegetative growth to be affected.

These results are not only highly promising regarding the long-term effectiveness of this particular biocontrol agent, but are also encouraging for future biocontrol programmes that require hemisphere translocations of biocontrol agents.

6. Supplementary Material

Data available from Figshare 10.6084/m9.figshare.13135877 (López-Núñez et al. 2021)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CrediT authorship contribution statement

HM, EM, RH and FI conceived the study. HM, EM, RH and FALN planned the experimental design. HM, LD, JP, EM and FALN collected data. FALN analysed the data and wrote the first draft of the manuscript with regular discussions and contributions from all co-authors. All co-authors revised and approved the final version of the manuscript.

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Figure captions

Figure 1- Sites of release and establishment of the biocontrol agent *Trichilogaster acaciaelongifoliae* between 2015 and 2019. The colours of the dots indicate the release year; solid dots indicate sites where establishment was confirmed in the following year. Numbers indicate regular monitoring sites: (1) São Jacinto dunes, (2) Quiaios, (3) Coimbra and (4) São Pedro de Moel. Coordinates of each site are available in Supplementary Material: Table S1.

Figure 2- Conceptual diagram of the experimental design used to evaluate the impact of *Trichilogaster acaciaelongifoliae* on *Acacia longifolia.*

Figure 3- (a) Female *Trichilogaster acaciaelongifoliae*; (b) Galled branches in different stages (with and without emergence holes); (c) *Acacia longifolia* heavily galled; (d) Timeline of release, detection and success ratio of the establishment of *T. acaciaelongifoliae* from 2015 (first release) to 2020 (last monitoring). Half-circles shown in green indicate the percentage of sites with confirmed *T. acaciaelongifoliae* establishment in each year-cycle (resulting from releases in the previous year). The length of the dotted-lines between release (green) and detection (black) indicate the duration of the life cycle, from oviposition to emergence. The maps at the bottom indicate the origin of the biocontrol agent released in each period.

Figure 4- (a) Spatial expansion of *Trichilogaster acaciaelongifoliae* population from 2016 to 2018 in the four regular-monitoring sites: São Jacinto dunes, Quiaios, Coimbra and São Pedro de Moel. Complementary information of site location can be found in Figure 1 and Supplementary Material: Table S1. Note the different scales in each aerial image. (b) Number of galls detected from 2016 to 2018, in spring and early-summer (March-July), in the four regular sites: São Jacinto dunes, Quiaios, Coimbra and São Pedro de Moel. The average number of galls per year is also shown. (c) Distances reached by *T. acaciaelongifoliae* in each year, from the release trees to the

most distant gall detected. In both (b) and (c), error bars represent standard error, n = 4. Letters show results of Tukey post-hoc test.

Figure 5- (a) Impacts of *Trichilogaster acaciaelongifoliae* on reproductive (number of seeds and pods) and vegetative (number of secondary branches and phyllodes, and total branch length) output of *Acacia longifolia*. The impacts are depicted as mean percentage change between the periods 2018-19 and 2019-20, across the three sites evaluated: São Jacinto dunes, Quiaos and São Pedro de Moel. Error bars show the standard error. Letters above bars show the results of a Tukey post-hoc test. (b) ununImpact of *T. acaciaelongifoliae* on reproductive and vegetative output of *Acacia longifolia* represented as the percentage of change observed in galled trees (calculated as the average of both galled and ungalled branches) in relation to reference values in ungalled trees.

Table 1- Detailed information about all release and monitoring campaigns along the Portuguese coast from 2015 to 2019. For each year, the number of *Trichilogaster acaciaelongifoliae* wasps released, the number of galls detected in the following year and the establishment rate (ratio between the number of detected galls and the released wasps) is shown.

Table 2- Number of imported, collected and emerged *Trichilogaster acaciaelongifoliae* individuals, as well as the number of release sites from 2015 to 2019.

Figures

Figure 1

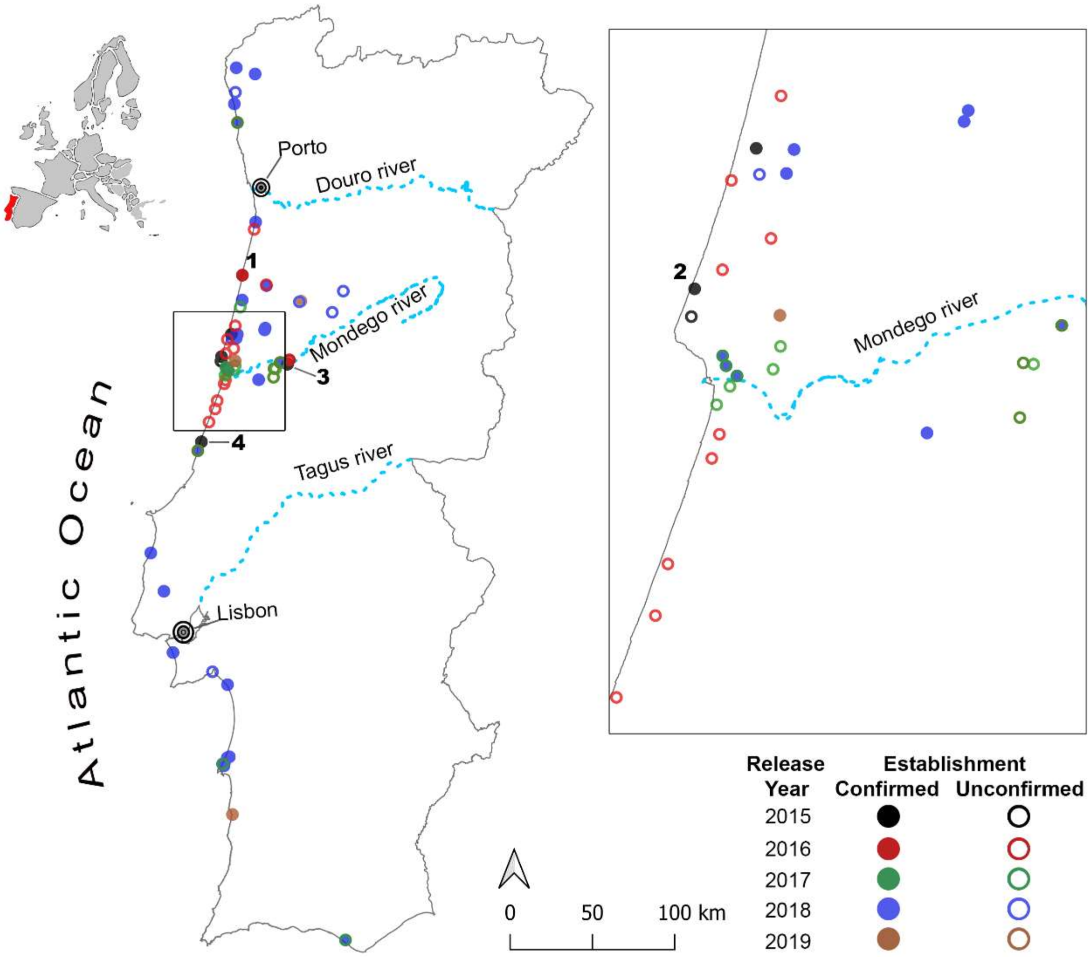
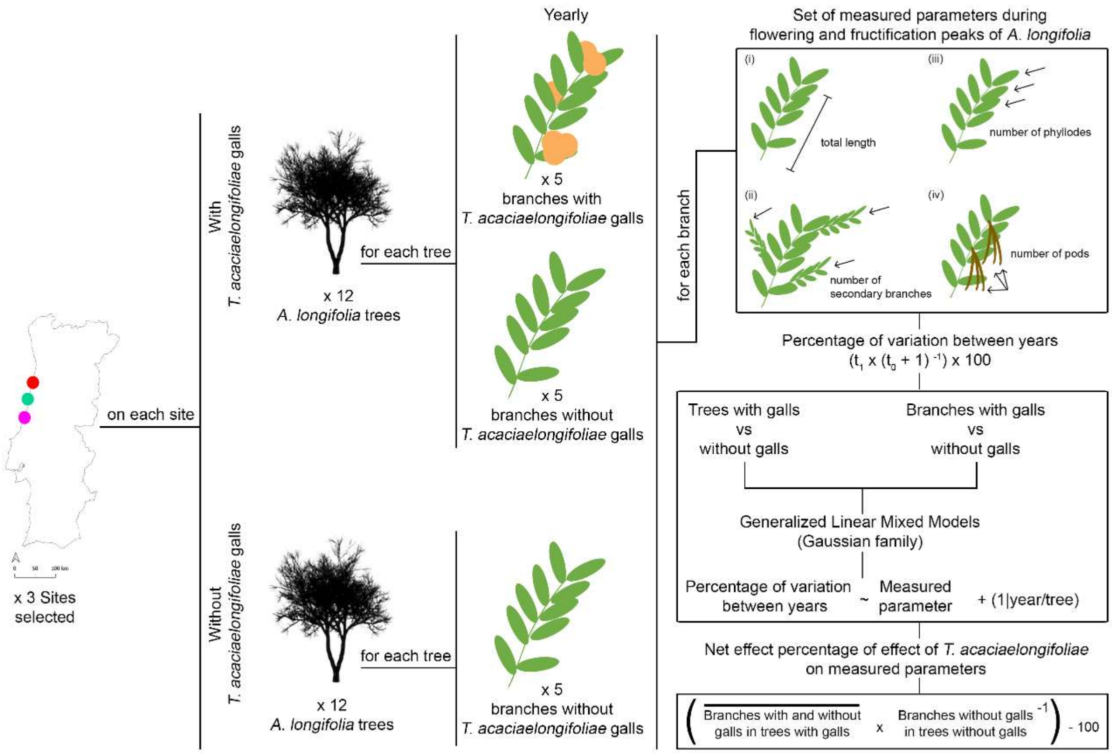

Porto
Douro river
Mondego river
Tagus river
Lisbon
Atlantic Ocean
1
2
3
4
Mondego river
0
50
100 km
Release Year
Establishment
Confirmed
Unconfirmed
2015
2016
2017
2018
2019

Figure 2



x 3 Sites
selected
on each site
With
T. acaciaelongifoliae galls
Without
T. acaciaelongifoliae galls
x 12
A. longifolia trees
for each tree
Yearly
x 5
branches with
T. acaciaelongifoliae galls
x 5
branches without
T. acaciaelongifoliae galls
for each branch
Set of measured parameters during
flowering and fructification peaks of A. longifolia
(i)
total length
(ii)
number of
secondary branches
(iii)
number of phyllodes
(iv)
number of pods
Percentage of variation between years
(t1 x (t0 + 1) -1) x 100
Trees with galls
vs
without galls
Branches with galls
vs
without galls
Generalized Linear Mixed Models
(Gaussian family)
Percentage of variation
between years
~
Measured
parameter
+ (1|year/tree)
Net effect percentage of effect of T. acaciaelongifoliae
on measured parameters
Branches with and without
galls in trees with galls
x
Branches without galls
in trees without galls
-1
- 100

Figure 3

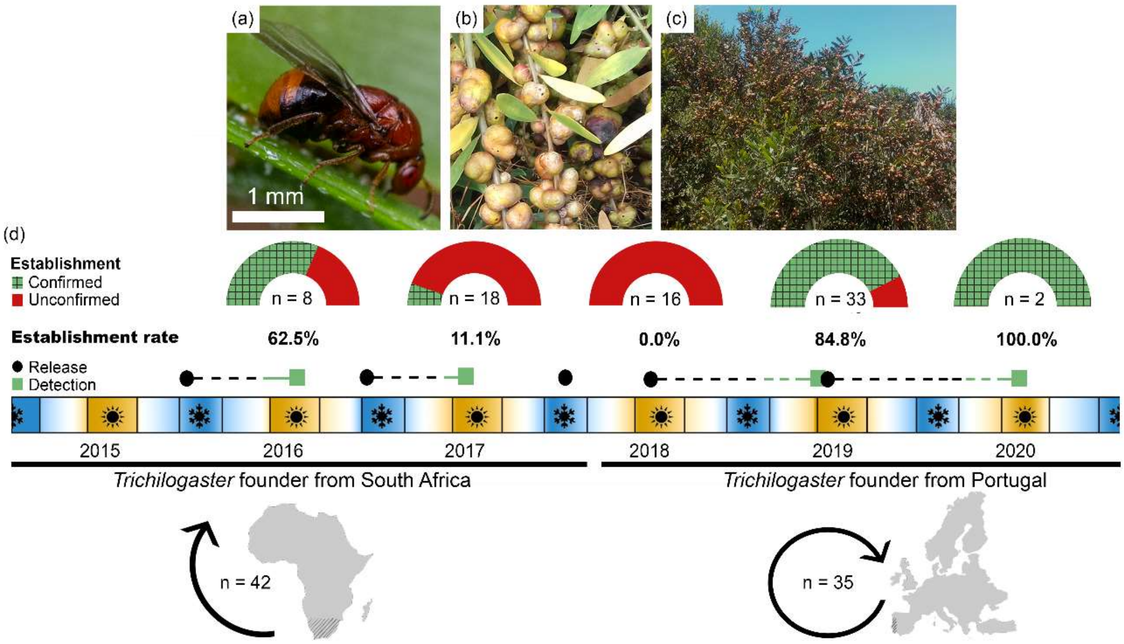

(a)
(b)
(c)
1 mm
(d)
Establishment
Confirmed
Unconfirmed
n = 8
n = 18
n = 16
n = 33
n = 2
Establishment rate
62.5%
11.1%
0.0%
84.8%
100.0%
Release
Detection
2015
2016
2017
2018
2019
2020
Trichilogaster founder from South Africa
Trichilogaster founder from Portugal
n = 42
n = 35

Figure 4

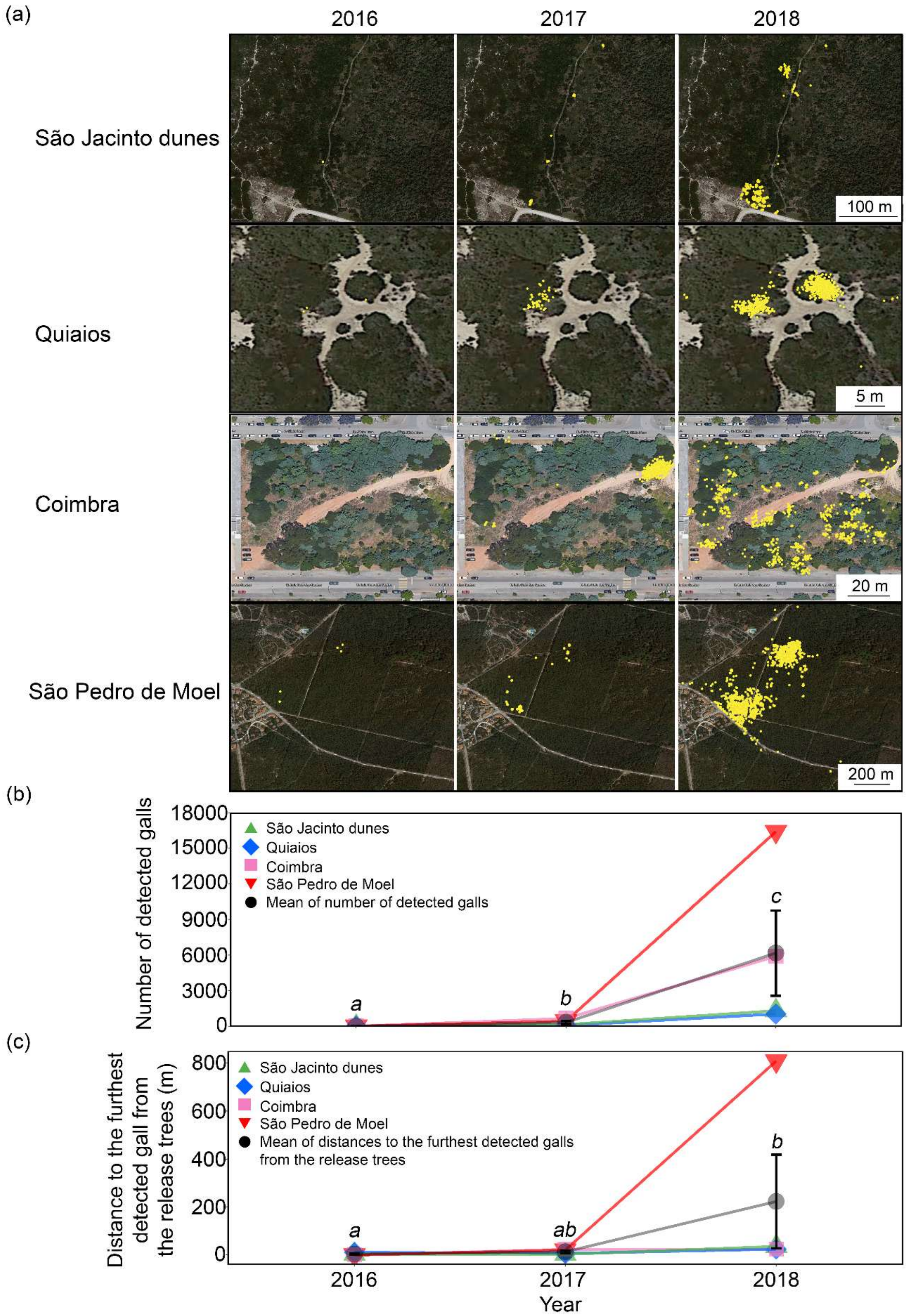
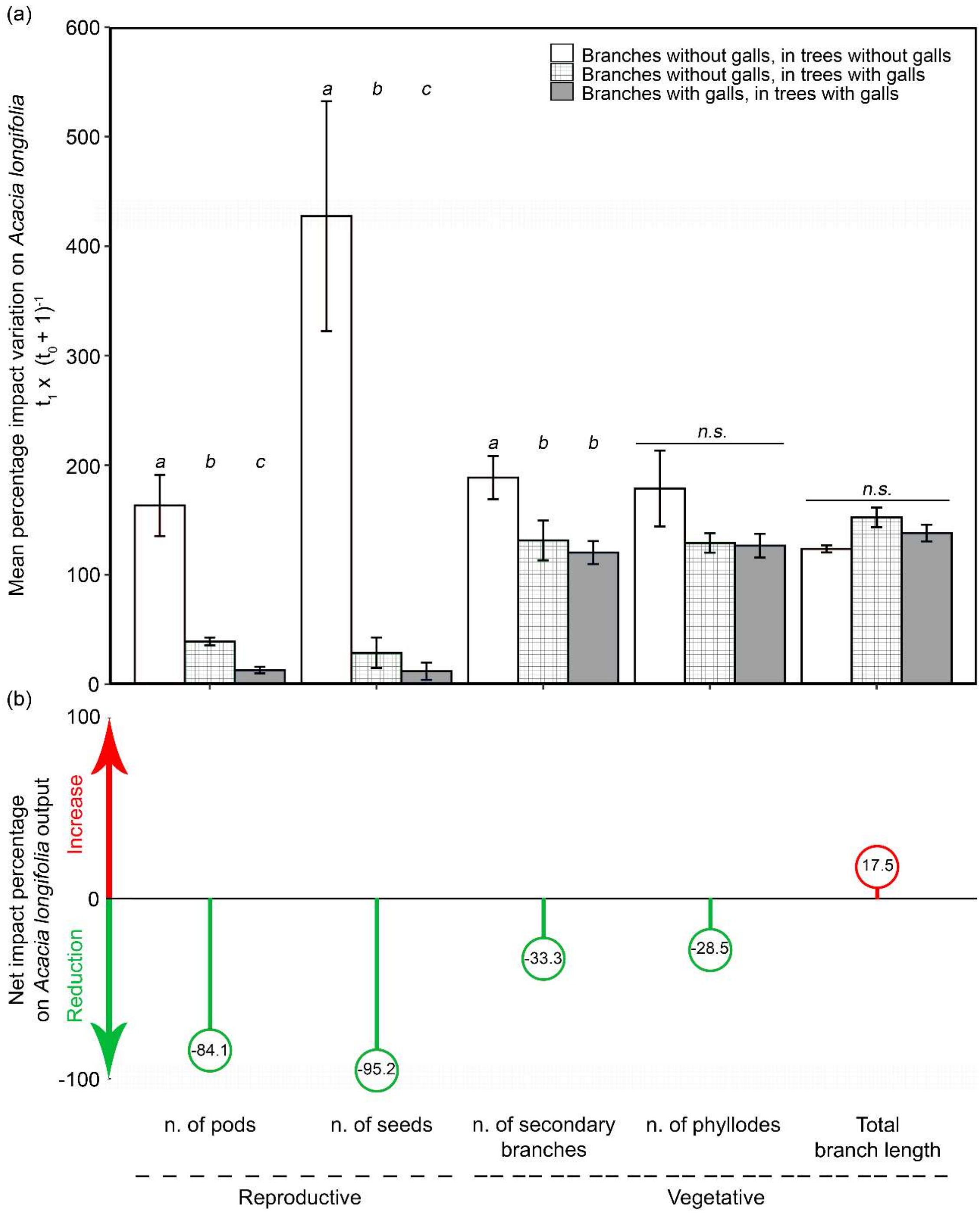

(a)
2016
2017
2018
São Jacinto dunes
Quiaios
Coimbra
São Pedro de Moel
100 m
5 m
20 m
200 m
(b)
Number of detected galls
18000
15000
12000
9000
6000
3000
0
São Jacinto dunes
Quiaios
Coimbra
São Pedro de Moel
Mean of number of detected galls
a
b
c
(c)
Distance to the furthest detected gall from the release trees (m)
800
600
400
200
0
São Jacinto dunes
Quiaios
Coimbra
São Pedro de Moel
Mean of distances to the furthest detected galls from the release trees
a
ab
b
2016
2017
2018
Year

Figure 5


(a)
Mean percentage impact variation on Acacia longifolia
t1 x (t0 + 1)-1
600
500
400
300
200
100
0
Branches without galls, in trees without galls
Branches without galls, in trees with galls
Branches with galls, in trees with galls
a b c
a b c
a b b
n.s.
n.s.
(b)
Net impact percentage on Acacia longifolia output
Increase
Reduction
100
0
-100
-84.1
-95.2
-33.3
-28.5
17.5
n. of pods
n. of seeds
n. of secondary branches
n. of phyllodes
Total branch length
Reproductive
Vegetative

Tables

Table 1

	Releases from South African galls									Releases from Portuguese galls					
	2015			2016			2017			2018			2019		
Site	Released	Detected	**Estab. Rate**	Released	Detected	**Estab. Rate**	Released	Detected	**Estab. Rate**	Released	Detected	**Estab. Rate**	Released	Detected	**Estab. Rate**
São Jacinto dunes[a]	88	1[b]	**0.011**	74	151	**2.041**	-	1317	**-**	-	0.0216[cd]	**-**	-	-	**-**
Quiaios[a]	80	9	**0.113**	-	73	**-**	-	1039	**-**	-	42.93[c]	**-**	-	-	**-**
Coimbra[a]	44	9	**0.205**	-	413	**-**	-	5899	**-**	-	298[c]	**-**	-	-	**-**
São Pedro de Moel[a]	74	9	**0.122**	-	437	**-**	-	16415	**-**	-	31.99[c]	**-**	-	-	**-**
Tocha	105	38	**0.362**	-	29	**-**	-	0[e]	**-**	-	-	**-**	-	-	**-**
Serra da Boa Viagem	65	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**
PN do Litoral Norte	30	0	**0.000**	139	0	**0**	39	0	**0.000**	83	2546[f]	**30.675**	-	-	**-**
Coimbra (Patos)	39	0	**0.000**	38	21	**0.553**	-	123	**-**	-	5544	**-**	-	-	**-**
Pinhal de Quiaios	-	-	**-**	75	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Tocha 1[e]	-	-	**-**	129	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Lagoas de Santo André	-	-	**-**	-	-	**-**	46	0	**0.000**	103	818	**7.942**	-	-	**-**
Vieira de Leiria[e]	-	-	**-**	76	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Faro	-	-	**-**	-	-	**-**	28	0	**0.000**	109	1117[f]	**10.248**	-	-	**-**
Seixo	-	-	**-**	-	-	**-**	46	0	**0.000**	-	-	**-**	-	-	**-**
Dunas de Vagos	-	-	**-**	-	-	**-**	-	-	**-**	146	733	**5.021**	-	-	**-**
Tocha 2	-	-	**-**	-	-	**-**	-	-	**-**	79	11	**0.139**	-	-	**-**
Tocha 3	-	-	**-**	-	-	**-**	-	-	**-**	9	0	**0.000**	-	-	**-**
Tocha 4	-	-	**-**	-	-	**-**	-	-	**-**	4	3	**0.750**	-	-	**-**
Lavos	-	-	**-**	66	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Leirosa	-	-	**-**	77	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Ovar[g]	-	-	**-**	135	0	**0.000**	-	0	**-**	-	-	**-**	-	-	**-**
Eixo	-	-	**-**	54	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Eixo 2	-	-	**-**	-	-	**-**	-	-	**-**	18	107	**5.944**	-	-	**-**
Paredes da Vitória	-	-	**-**	138	0	**0.000**	21	0	**0.000**	77	67	**0.870**	-	-	**-**
Mata do Urso[e]	-	-	**-**	89	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Mira[e]	-	-	**-**	45	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Condeixa	-	-	**-**	5	0	**0.000**	2	0	**0.000**	-	0	**-**	-	-	**-**
Pedrogão[e]	-	-	**-**	45	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Lagoa da Vela[e]	-	-	**-**	70	0	**0.000**	-	-	**-**	-	-	**-**	-	-	**-**
Covões	-	-	**-**	37	0	**0.000**	69	0[h]	**0.000**	2	3	**1.500**	-	-	**-**
Anobra	-	-	**-**	21	0	**0.000**	6	0	**0.000**	-	-	**-**	-	-	**-**
Anobra 1[h]	-	-	**-**	-	-	**-**	20	0	**0.000**	-	-	**-**	-	-	**-**
Figueira da Foz	-	-	**-**	-	-	**-**	48	0	**0.000**	12	5	**0.417**	-	-	**-**
Figueira da Foz 1	-	-	**-**	-	-	**-**	22	0	**0.000**	16	81	**5.063**	-	-	**-**
Figueira da Foz 2	-	-	**-**	-	-	**-**	6	0	**0.000**	8	31	**3.875**	-	-	**-**

Vila Verde[h]	-	-	**-**	-	-	**-**	16	0	**0.000**	-	-	**-**	-	-	**-**
Gala	-	-	**-**	-	-	**-**	10	0	**0.000**	-	-	**-**	-	-	**-**
Morraceira	-	-	**-**	-	-	**-**	8	0	**0.000**	-	-	**-**	-	-	**-**
Alhadas[h]	-	-	**-**	-	-	**-**	6	0	**0.000**	-	-	**-**	-	-	**-**
Antas	-	-	**-**	-	-	**-**	-	-	**-**	54	51	**0.944**	-	-	**-**
Monte Feio – Sines 1	-	-	**-**	-	-	**-**	-	-	**-**	16	6	**0.375**	-	-	**-**
Monte Feio – Sines 2	-	-	**-**	-	-	**-**	-	-	**-**	20	2	**0.100**	-	-	**-**
Pesqueiro Sancha	-	-	**-**	-	-	**-**	-	-	**-**	52	11	**0.212**	-	-	**-**
Tróia – Comporta road	-	-	**-**	-	-	**-**	-	-	**-**	46	23	**0.500**	-	-	**-**
Praia do Navio, Santa Cruz	-	-	**-**	-	-	**-**	-	-	**-**	13	5	**0.385**	-	-	**-**
Setúbal beach road	-	-	**-**	-	-	**-**	-	-	**-**	12	0	**0.000**	-	-	**-**
Barrinha de Esmoriz	-	-	**-**	-	-	**-**	-	-	**-**	45	77	**1.711**	-	-	**-**
Anha	-	-	**-**	-	-	**-**	-	-	**-**	9	0	**0.000**	-	-	**-**
Quinta Pentieiros	-	-	**-**	-	-	**-**	-	-	**-**	25	26	**1.040**	-	-	**-**
Caparica beaches	-	-	**-**	-	-	**-**	-	-	**-**	48	77	**1.604**	-	-	**-**
Breijinhos	-	-	**-**	-	-	**-**	-	-	**-**	43	321	**7.465**	-	-	**-**
Soure	-	-	**-**	-	-	**-**	-	-	**-**	45	35	**0.777**	-	-	**-**
Belazaima do Chão	-	-	**-**	-	-	**-**	-	-	**-**	20	3[i]	**0.150**	-	-	**-**
Pocariça 1	-	-	**-**	-	-	**-**	-	-	**-**	39	37	**0.949**	-	-	**-**
Pocariça 2	-	-	**-**	-	-	**-**	-	-	**-**	21	39	**1.857**	-	-	**-**
Carapinheira, Mafra	-	-	**-**	-	-	**-**	-	-	**-**	34	25	**0.735**	-	-	**-**
IP3 road access	-	-	**-**	-	-	**-**	-	-	**-**	3	0	**0.000**	-	-	**-**
Vila Nova da Rainha	-	-	**-**	-	-	**-**	-	-	**-**	3	0	**0.000**	-	-	**-**
Riba de Âncora	-	-	**-**	-	-	**-**	-	-	**-**	48	153	**3.188**	-	-	**-**
Vila Nova de Mil Fontes	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	24[j]	4	**0.166**
Alhadas quarry	-	-	**-**	-	-	**-**	-	-	**-**	-	-	**-**	50[j]	8	**0.160**

[a] These 4 sites are regularly monitored in more detail; [b] Detected 1 dried gall in 10/07/2017 monitoring resulting probably from the 2015 release campaign; [c] Estimated gall density gall/m^2); [d] Estimated using two grids of 100 x 200 m; [e] This site burned in 2017; [f] Although we were not able to detect galls in the previous years, this high number of galls suggest that there was establishment in one of the previous releases; [g] Many tagged *Acacia longifolia* were cut around this area in 2018; [h] Acacias were cut; [i] 3 galls detected in 06/06/2020 resulting probably from the 2018 release campaign; [j] In this campaign galls were left in the field instead of wasps, these numbers assume that one wasp emerged per gall. The dash indicates an absence of monitoring or release planning for the site.

Table 2

	2015	2016	2017	2018	2019	2020	Total
# Galls imported from South Africa	1400	1276	397	-	-	-	3073
# Galls collected from Portugal	-	0	0	1091	73[e]	-	1164
# *T. acaciaelongifoliae* females emerged	-[a]	1480	581	1546	-[f]	-	3607
# *T. acaciaelongifoliae* males emerged	-[a]	231	35	2	-[f]	-	268
# *T. acaciaelongifoliae* released	525[b]	1313[b]	393[b]	1262[c]	74[cg]	-	3567
# Release sites	8	18	16	33	2	-	61[d]
# Detected galls	-	66	1124	24793	11954[i]	12[j]	37949[k]

[a] Not counted; [b] Wasps emerged from South African galls; [c] Wasps emerged from Portuguese galls; [d] In several sites wasps were released in more than one year since there was no establishment in the previous year; as such, the total of releases is greater than the actual number of physical sites; [e] 50 of these galls had emergence holes; [f] Galls released in the field instead of wasps, emerging insects not counted; [g] Galls released in the field we assumed 1 wasp emerged per gall; [i] Galls counted only in sites where releases occurred until 2018; [j] Galls counted only in sites where released occurred in 2019; [k] This is an underestimation as from 2018 onwards galls were no longer counted in sites where releases occurred in 2015 and 2017. Dashes indicates absence of data.