J. Sher-e-Bangla Agric. Univ., 14(1&2): 69-77, January & July 2023 ISSN 1997-6038

THE EFFECT OF GALLS FORMED BY TRICHILOGASTER ACACIAELONGIFOLIAE ON THE VEGETATIVE GROWTH OF INVASIVE ACACIA LONGIFOLIA SUBSPECIES LONGIFOLIA IN AUSTRALIA

M. R. Islam[1*], W. Wright[2], G. Palmer[3] and H. Hira[4]

ABSTRACT

A study was conducted to observe the effectiveness of galls by *Trichilogaster acaciaelongifoliae* on the vegetative growth of *Acacia longifolia* subspecies *longifolia* at six study locations in the Greater Grampians (GGr) Bioregion of Victoria, Australia during a study period from September 2014–December 2016. The results of this study have showed that galls formed by *Trichilogaster acaciaelongifoliae* on *Acacia longifolia* subspecies *longifolia* plants have negative impacts on vegetative growth of *Acacia longifolia* subspecies *longifolia* growingin the native home range of the weed, however it is not sufficient to reduce the invasive spread of the plant. An integrated weed management approach is required to control the invasive weed *Acacia longifolia* subspecies *longifolia*.

Keywords: Infestation intensity, phyllodes, sub-branches, environmental weed, biological control

INTRODUCTION

Trichilogaster acaciaelongifoliae (Froggatt) (*T. acaciaelongifoliae*) is a hymenopteran wasp, origin in Australia found in the galls on *Acacia longifolia* subspecies *longifolia* (*A. l. longifolia*) (Dennill *et al.,* 1999). Haiden *et al.* 2012 explains that galls develop because a stimulus from the insect alters normal physiological processes within the plant. The redirection of resources associated with the formation of galls impair vegetative growth of the host plant (Fernandes *et al.,* 2012). For example, in a case study from South Africa, *Trichilogaster signiventris* causes the development of galls on vegetative parts of *Acacia pycnantha.* Normal growth of the plant is hampered by the redirection of plant resources (Dorchin *et al.,* 2006).

Among the numerous examples of negative effects on plant growth, due to the formation of galls is a study by Klöppel *et al.* (2003), which showed that galls formed by the cynipid wasp, *Aulacidea subterminalis*, have a significant impact on growth of the grassland weed *Hieracium pilosella*. The negative effects of galls on plant growth and reproduction have been successfully used to help control pest plants in some instances. The eurytomid wasp *Eurytoma attiva* (Burks) has been successfully used to control black sage, *Cordia curassavica* Jacq. (R & S) in Mauritius as it damages the reproductive parts of the plant and prevents reinvasion of the weed in areas that have been difficult to manage the weed. This gall-forming wasp has also been used successfully in Malaysia and Sri Lanka to control the black sage (Cock *et al.,* 1985).

Biological control of weeds can be an effective strategy for long-term restoration of native ecosystems (Richardson and Kluge, 2008; Wilson *et al.,* 2011). *T. acaciaelongifoliae* has been applied as a biological control agent to manage introduced, invasive *A. l. longifolia* populations in South Africa (Hoffmann *et al.,* 2002), where both the wasp and the plant are introduced.

A. l. longifolia is regarded as a significant environmental weed in its native distribution in several Australian states (Luke *et al.,* 2008; Thomson, 2016; Milkins, 2017), where it co-occurs naturally with *T. acaciaelongifoliae,* While it is apparent that the wasp is not acting to control *A. l. longiofolia* in these areas- (since populations of the plant continue to spread) (Milkins, 2017), it is not understood whether this is because galls formed by the wasp do not negatively affect the plant in its native home range.

[1*] Correspondence author & Associate Professor, Dep. of Entomology, Sher-e-Bangla Agricultural University and PhD Fellow, Environmental Science, Federation University Australia, [2] Professor, Conservation Biology, [3] Senior Lecturer, [4] PhD Fellow and Research Associate, Environmental Science, Federation University Australia.

Therefore, the aim of this study is to test whether reduced vegetative growth in *A. l. longifolia* occurs due to galls formed by *T. acaciaelongifoliae* in the Australian context.

MATERIALS AND METHODS

Study area

Measurements of the growth of *A. l. longifolia* were made at six study locations in the Greater Grampians (GGr) Bioregion of Victoria, Australia during a study period from September 2014–December 2016, where PP1 = Public Property 1, PP2 = Public Property 2, PP3 = Public Property 3, PV4 = Parks Victoria 4, PV5 = Parks Victoria 5 and PV6 = Parks Victoria 6.

Data collection

The methods for data collection and analysis were derived by some modification of methods used by Impson *et al*. (2013) and Hartnett and Abrahamson (1979).

Infestation intensity

The intensity of infestation of *A. l. longifolia* plants by *T. acaciaelongifoliae* was assessed once, at the beginning of the study period in September 2014. The intensity of infestation was calculated by positioning a 25m^2 quadrat (considering the plant density and plant canopy of *A.l. longifolia* in the study locations) pseudo-randomly within each of the six study locations and assessing the number of *A. l. longifolia* plants present within the quadrat, noting how many plants bore galls. Infestation intensity was calculated as the number of *A. l. longifolia* plants bearing galls divided by the total number of *A. l. longifolia* plants present in each (5x5) = 25 m^2 area; expressed as a percentage.

Vegetative growth

In order to understand the effects of the presence of galls on the growth of *A. l. longifolia*, three galled and three un-galled mature *A. l. longifolia* plants were selected at of the six study locations (*N*=36). One branch of each selected tree was tagged and several parameters were measured on these branches at monthly intervals throughout the study period to provide proxy measures of growth of *A. l. longifolia*. Each branch was considered to be comprised of many (usually 3-12) sub-branches and was chosen based on size (1.5 to 2.5 m long). Initially, the length of galled and ungalled branches were roughly equal in size. For several parameters, sub-branches were used instead of branches as this was more practical and provided a larger data set for analysis.

Branch and sub-branch length

Plant growth parameters measured were: Branch and sub-branch length: measured every month for the first year but only every second month for the second year of the study due to consistent trends of the data.

Phyllodes

Phyllodes serve the same function as the leaves of other plants (i.e. photosynthesis). As in other members of the *Acacia* genus, the petioles of *A. l. longifolia*, are flattened and widened to form leaf-like structures known as phyllodes (Plate 3). Anatomically, leaves comprise lamina, petiole and leaf base. In Acacias, the modified petiole performs as leaf. Number of phyllodes per sub-branch: measured every month throughout the first year of the study. The effect of galls on the vegetative of *A. l. longifolia* was assessed by comparing the parameters described above for galled and ungalled trees.

Data analysis

All data analysis was conducted using SPSS Statistics Version 22, with reference to Allen *et al.* (2014). A chi-squared test was performed to assess whether infestation intensity differed significantly across locations. Assumptions of independence and expected frequencies were checked and found not to have been violated (Allen *et al.,* 2014).

RESULTS AND DISCUSSION

Infestation intensity

Infestation of *A. l. longifolia* by *T. acaciaelongifoliae* was observed across all study locations at the beginning of the study period. The highest proportion of infested trees (83.3%) was found at location

PP1 (Table 1), a private property densely covered by *A. l. longifolia* plants known to be more than three years of age. The lowest proportion of infested trees (42.9%) was found at location PP2 (Table 1).

Table 1. Percent infestation of *A. l. longifolia* by *T. acaciaelogifoliae* in 25m^2 quadrats assessed at six study locations in the Greater Grampians Bioregion, Victoria, Australia in September 2014.

Locations	Total number of *A. l. longifolia* plants in 25 m^2	Number of *A. l. longifolia* plants infested by *T. acaciaelongifoliae* in 25 m^2	Percent infestation (%) of *A. l. longifolia* plants by *T. acaciaelongifoliae* in 25 m^2
PP1	18	15	83.3
PP2	14	6	42.9
PP3	9	5	55.6
PV4	15	9	60.0
PV5	12	9	75.0
PV6	11	6	54.5

The proportion of trees affected by *T. acaciaelongifoliae* was more than 50% at all four other study locations; and the chi-squared test indicated no significant differences in infestation intensity between the locations (p=1.00). With the exception of PP1, all locations were covered by a mix of *A. l. longifolia* and other native vegetation; locations PV4 and PV5 had been recently invaded by *A. l. longifolia* plants, following a fire in 2014 (Milkins, 2017) enhanced germination of the *A. l. longifolia* seeds. Its spread has impeded the germination and growth of other native plants in these locations. *A. l. longifolia* trees with more branches and more flower buds may provide greater opportunities for *T. acaciaelongifoliae* infestation (PP1) since these structures are thought to be preferred by the wasp for egg deposition. *T. acaciaelongifoliae* shows a preference for flower or twig buds on younger branches of *A. l. longifolia* plants for egg deposition (Islam, 2022). Such branches are more common at locations PV4 and PV5 (Plate 1).

(A) (B)

Plate 1. Abundance of younger branches of *A. l. longifolia* in the study locations PV4 (A) and PV5 (B).

The impact of galls on growth

The impact of galls formed by *T. acaciaelongifoliae* on the vegetative growth of *A. l. longifolia* was evaluated by measuring sub-branch length (growth rate) per branch and numbers of phyllodes per sub-branch at six different locations throughout the study period and comparing these measures for galled and ungalled branches.

Plate 2. Sub-branches of *A. l. longifolia* with galls (inset photo of gall at a month of age) formed by *T. acaciaelongifoliae* (white colour) and without galls (red colour) in the study location in the Greater Grampians Bioregion, Victoria, Australia.

The effects of galls on the length of sub-branches

Initially, the average length of galled and ungalled branches of *A. l. longifolia* were roughly equal in size (Plate 2), however, the average length of ungalled branches increased over the time compare to galled branches (Fig. 1).

The average length of galled sub-branches (mean length, M=94.98 cm; standard deviation, SD=13.63 cm) were significantly shorter than ungalled sub-branches (mean length, M=117.37 cm; standard deviation, SD=25.9 cm) of *A. l. longifolia* at the end of the study F(1, 24)=47.39, p<.005, partial η^2=.66. The partial η^2, indicates that the presence of galls has a large effect on the length of galled sub-branches (Fig.1) at different locations. However, there were no significant effects of location on the length of sub-branches F(5, 24)=1.87, p=.137, partial η^2=.281 and no significant interaction between treatments (galled/ungalled) and locations *F*(5, 24)=0.364, *p*=.868, partial η^2=.071.

Not surprisingly, time (the months of the study period) explains a great deal of the variation in the lengths of both galled and ungalled sub-branches (galled and ungalled) of *A. l. longifolia*. All sub-branches increased in length over time *F*(17, 408)=2202.55, *p*<.001, partial η^2=.99), however galled branches increased at a slower rate than ungalled branches F(1, 24)=47.39, p<.005, partial η^2=.66). A slower growth rate in galled sub-branches is implied by a significant combined effect of time and the presence of galls on the length of sub-branches F(17, 408)=563.01, p<.001, partial η^2=.96. The effect size of changes in the length of the sub-branches over time was almost five times higher (η^2 =.77) in ungalled branches than galled branches (η^2 =.15) of *A. l. longifolia* (Fig. 1).

Seasonal differences in growth were also evident; and were also influenced by the presence of galls. Mean lengths of galled sub-branches did not differ significantly from their initial lengths (measured in September 2014) until after January 2015 when season growth began (Post hoc comparisons using the LSD test with α=.05; M=90.95 cm, SD=11.31 cm).

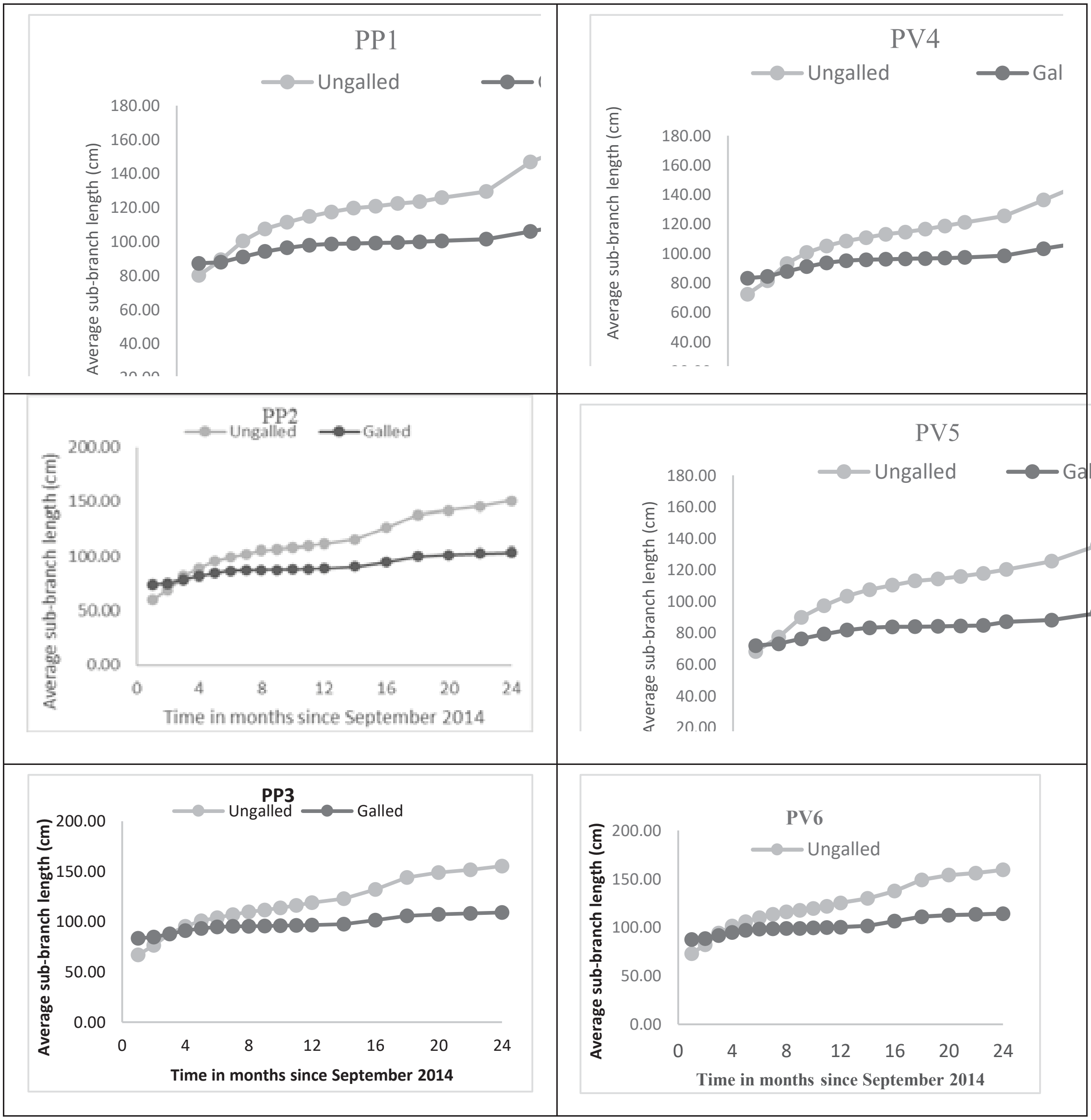

Fig. 1. **Monthly variations in the average length of sub-branches of *A. l. longifolia* with galls formed by *T. acaciaelongifoliae* and of un-galled sub-branches during a two-year period from September 2014 to August 2016 at six different study locations (1-6, PP= private property, PV= Parks Victoria and indicates the Grampians National Park) in the Greater Grampians Bioregion, Victoria, Australia..**

The galls produced by *T. acaciaelongifoliae* reduced the vegetative growth of *A. l. longifolia*. Although galled sub-branches consistently showed lower growth rates than ungalled sub-branches, the extent of the difference in growth rate between galled and ungalled sub-branches varied across the six locations. Location PP1 supported older stands of *A. l. longifolia* compared to other locations (although there were some younger *A. l. longifolia* present). The initial (measured in September 2014) average length, of galled sub-branches at location PP1 was 87.59cm (Fig. 1). These galled sub-branches increased their average length by only 26.57 cm over the two-year study period (Fig. 1). On the other hand, the initial average length of un-galled sub-branches at location PP1 was 72.89 cm. These branches grew at a rate of approximately three times higher than galled branches in the same location, reaching an average length of 159.40 cm by August 2016 (Fig. 1).

The greatest differences in length between galled and ungalled sub-branches were found at locations PV5 and PV4, (57.6 cm and 53.90 cm) respectively, where younger *A. l. longifolia* were the most common. It is possible that the age of the plant may influence the rate of sub-branch growth; this was not tested here, and there was no bias in the age of plants selected for galled and ungalled groups in this study. Galled branches grow more slowly than those without galls and the reduced growth rate observed in galled branches is likely to be due to galls acting as nutrient sinks (Dennill, 1985). Such effects have also been shown for other insect-plant relationships. For example, Shaw *et al.* (2009) showed that a psyllid, *Aphalara itadori* Shinji reduced vegetative growth of *Fallopia japonica* (Houtt) in the UK. *Fallopia japonica* is an invasive weed in the UK, North America and greater Europe.

The effect of galls on number of phyllodes

The number of phyllodes per sub-branch of *A. l. longifolia* did not differ significantly across the six study locations ($F(5, 24)=0.77$, $p=.58$, partial $\eta^2=0.14$).

Plate 3. Phyllodes on the sub-branch of *A. l. longifolia* act as leaves in the photosynthetic process.

There was no significant interaction between treatment (presence/absence of galls) and locations ($F(5, 24)=.79$, $p=.56$, partial $\eta^2 = 0.14$); however the presence of galls had an effect on the number of phyllodes per sub-branch of *A. l. longifolia* $F(1, 24)=322.64$, $p<.001$, partial $\eta^2=0.93$. Considering all measurements made over a one year period, the average number of phyllodes per sub-branch of *A. l. longifolia* was 1.7 times higher in ungalled sub-branches (M=26.44, SD=1.38) compared to galled sub-branches (M=16.00, SD=2.91). More phyllodes per ungalled sub-branch allowed more photosynthesis and the production of more energy resources for further growth of the plant (Plate 3).

Initially, the difference between the mean number of phyllodes per sub-branch for galled and ungalled sub-branches of *A. l. longifolia* was four, increasing to 28 after one full year of study (Fig. 2).

The number of phyllodes per sub-branch increased over time for both galled and ungalled plants. Post hoc comparisons using the LSD test revealed, for ungalled plants, differences between the initial number of phyllodes per sub-branch (measured in September 2014; M=26.44, SD=1.38) and the number of phyllodes per sub-branch measured in April 2015 (M=37.00, SD=2.72). During this period, *A. l. longifolia* starts growing rapidly in response to weather factors. However, there was no significant difference between the initial number of phyllodes (M=19.00, SD=4.24) on sub-branches of galled *A. l. longifolia* compared to the number measured in April 2015 (M=16.00, SD=2.91) (Fig. 2). The gall inducing wasps emerge from galls and galls dessicate during this period. After the month of April, the number of phyllodes increase in both cases due to weather factors, however it was comparatively low in number in galled branches than ungalled branches. Thus galled sub-branches did not increase their capacity for photosynthesis over time compared to ungalled sub-branches.

A combined effect of the presence of galls and time on the number of phyllodes per sub-branch of *A. l. longifolia* was found $F(11, 264) = 0.375$, $p<.001$, partial $\eta^2 = 0.85$. There is a difference in the average number of phyllodes over time. The highest number of phyllodes was found in the month of August 2015, which was followed by July and June 2015. However, there was no significant interaction between time and locations $F(55, 264) = 0.38$, $p=1.00$, partial $\eta^2 = 0.07$; nor was there a significant interaction among time, treatments and locations $F(55, 264) = 0.64$, $p = 0.98$, partial $\eta^2 = 0.12$ on the number of phyllodes of *A. l. longifolia*. The number of phyllodes per sub-branch of *A. l. longifolia* was affected by the presence of galls , as was the rate of increase in the number of phyllodes over time. It is likely that a reduced number of phyllodes in galled sub-branches has follow-on effects, possibly including reductions in other growth parameters, and in reproductive success.

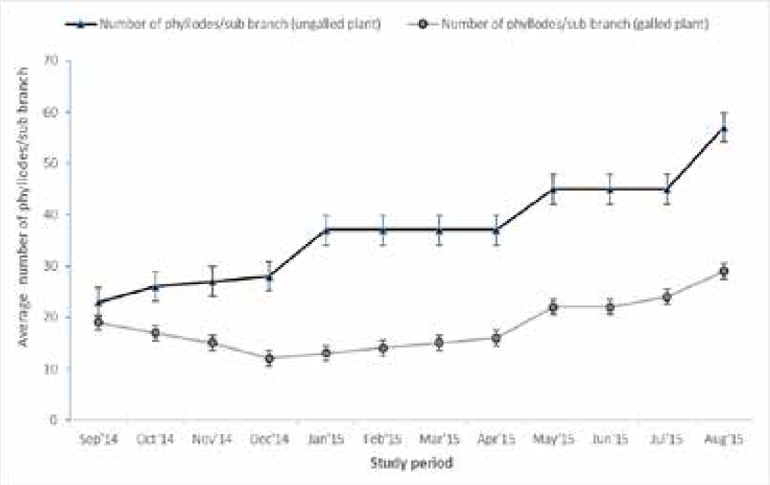

Fig. 2. Number of phyllodes on galled (*T. acaciaeloguifoliae)* and ungalled sub-branches of *A. l. longifolia* from September 2014-August 2015.

Initially, the difference between the mean number of phyllodes per sub-branch for galled and ungalled sub-branches of *A. l. longifolia* was four, increasing to 28 after one full year of study (Fig. 2). The average number of phyllodes per sub-branch was significantly affected by the presence of galls over the time of study period. There was some indication of seasonal variability in the average number of phyllodes per sub-branch.

The average number of phyllodes per sub-branch on galled *A. l. longifolia* plants decreased gradually from September 2014 to December 2014. The number of phyllodes per branch on galled branches then remained similar from January 2015 until April 2015 (Fig. 2). At this time of year, the adult insects emerge from the galls; and the galls start to desiccate; and are no longer redirecting the plants' resources. The number of phyllodes per galled sub-branch increased in May 2015 but remained lower than in ungalled sub-branches. The number of phyllodes per sub-branch on ungalled sub-branches increased steadily over the period of the study (Fig. 2).

The initial average number of phyllodes per sub-branch in September 2014 was 23 (ungalled) and 19 (galled). These numbers increased to 57 (ungalled) and 29 (galled) per sub-branch in August 2015 after one year (Fig. 2). The galls have the potential to accumulate nutrients, which would otherwise be directed into the formation of phyllodes. Dennill (1985) observed similar results in South Africa, suggesting that galls have an adverse effect on the number of phyllodes. Galls formed by *Dryocosmus kuriphihs,* a cynipid wasp, are known to significantly reduce the vegetative growth of the chestnut tree in Japan (Kato and Hijii, 1997).

However, some galls (by *T. acaciaelongifoliae*) bearing branches (*A. l. longifolia*) in the study location still able to grow and produce a number of flowers and seeds, which invades more areas in the following year. Performance of biological control agent can vary by spatial and temporal changes with abiotic and biotic conditions such plants resources, soil ferltility, weather factors, increased resistance of the weed, natural enemies (such as bird, predatory insects, parasitoids) of the biocontrol agent (Denno *et al.,* 2002, Stiling and Moon, 2005, Hovick and Carson, 2015). So that reliance only on *T. acaciaelongifoliae* as a biological control of *A. l. longifolia* is not effective in Australia. The high abundance and persistence of *A. l. longifolia* seeds in the soil seed bank is likely to further reduce the effectiveness of *T. acaciaelongifoliae* as a sole control agent. Impson *et al.* (2013) also found similar results when considering the use of the gall-forming midge *Dasineura rubiformis* (Cecidomyiidae) to control *Acacia mearnsii* (black wattle) and recommended the use of the midge as part of an integrated appraoch to weed control. Thus an integrated management including *T. acaciaelongifoliae* might be effective to control *A. l. longifolia* in the native range.

CONCLUSION

This research has demonstrated that galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* plants reduce vegetative growth of *A. l. longifolia* growing in the native home range of both species, although this does not appear to be sufficient to reduce the invasive spread of the plant. It is concluded that effective control of the invasive weed *A. l. longifolia* will require an integrated weed management approach and recognise that while insufficient as a sole control agent, the role of *T. acaciaelongifoliae* in reducing vegetative and reproductive growth is likley to be an important component of an integrated approach.

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