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RESEARCH

Compromised compensation: evaluating the fitness costs of tolerance responses in plants facing herbivore-induced delayed germination and intraspecific competition

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Background: Many plants compensate for the damage caused by herbivorous insects through tolerance responses. Besides directly causing plant tissue loss and seed production reduction, herbivory causes phenological changes in the host plant. However, little is known about the fitness costs of phenological changes caused by tolerance responses to herbivorous attacks.
Results: The girdling beetle *Phytoecia rufiventris* caused a short-term decrease in the number of flowers of the host plant *Erigeron annuus*. However, accelerated growth restored the number of flowers, but after a 2-week delay. With an objective to examine whether the tolerance response with such a delay fully compensates the fitness, we experimentally reproduced a 2-week delay in germination under greenhouse and field settings. Under both conditions, intraspecific competition resulted in serious defects in the growth and reproduction of *E. annuus* plants which of germination was delayed. However, delayed germination (DG) resulted in better growth when competition and herbivory were eliminated from the field. Thus, we showed that the tolerance response to restore reproductive production does not fully compensate for the fitness loss caused by insect attack; rather, the delay in seed production in attacked plants leads to DG and subsequent inferiority in intraspecific competition.
Conclusions: Our results imply that compensation for floral production after an herbivore attack does not fully restore offspring fitness in the presence of intraspecific competition and herbivory. Assessing the ecological consequences of defense traits in an appropriate layer of interaction is critical to interpreting adaptive values.

Keywords: competition, Darwinian fitness, *Erigeron annuus*, phenological shift, *Phytoecia rufiventris*, plant-herbivore interaction, tolerance response

Introduction

Plants attacked by insect herbivores defend themselves to minimize fitness loss by the attack (Erb 2018). While the herbivore attack is blocked by resistance traits (e.g., Plant secondary metabolites [Fraenkel 1959], protease inhibitors [Hartl et al. 2010], and herbivore-induced plant volatiles [Turlings and Benrey 1998]), the damage is restored by tolerance traits (Strauss and Agrawal 1999). Tolerance allows plants to grow and reproduce under herbivore attack. Specifically, insect attacks induce an increase in photosynthetic rate (Halitschke et al. 2011) and compensatory growth (Mcnaughton 1983) which overcome the loss of tissue by herbivory. Especially, damage to the apical meristem releases the apical dominance and induces branching which leads to increased production of the reproductive organ (Paige and Whitham 1987). Attacked plants also reallocate resources from damaged organs to less vulnerable organs and boost proliferation to restore damage (Schwachtje et al. 2006). The tolerance responses of plants that are often sufficient to recover from damage may even overcompensate for the performance of undamaged plants (Agrawal 2000).

Although tolerance response is generally accepted as adaptive, the degree to which the damage is restored varies. The mode of attack (Strauss and Agrawal 1999), nutritional state (Mutikainen and Walls 1995), abiotic factors

(Maschinski and Whitham 1989), and neighbors in the same community (Maschinski and Whitham 1989) affect the extent of damage compensation in the attacked plant. Thus, the adaptive value of tolerance response may differ depending on context. It is important to understand both the cost and benefit of one trait to evaluate the fitness value of the trait. While there are several mechanisms studied that exert costs on tolerating plants (e.g., increased herbivory [Utsumi and Ohgushi 2009]), what factors hinder tolerating plants from restoring fitness is less studied compared to the apparent benefit of tolerance response.

The difference in fitness between damaged and undamaged plants is a typical measure of tolerance. While it is the most precise way to measure fitness following the definition-contribution to the gene pool of the next generation-, the direct measurements of fitness are often replaced by parameters such as plant damage (Steppuhn et al. 2004), plant growth (Lehndal and Ågren 2015), and the number of flowers (Schuman et al. 2012) and seeds (Baldwin 1998). Notably, the extent to which tolerance restores fitness depends on the fitness components used to measure the tolerance. For instance, male and female fitness is differentially restored after herbivore attack (Agrawal et al. 1999) while fruit and seed production display different outcomes of tolerance (Banta et al. 2010). Moreover, reproductive tolerance can show a trade-off with vegetative tolerance (Wise and Mudrak 2021). The inconsistency of multiple parameters restricts our understanding of the adaptive value of tolerance responses. Though measuring the value of tolerance at the offspring growth would be important to evaluate the adaptive value of tolerance, growth and reproduction of offspring produced by tolerating and undamaged plants have not been intensively investigated.

A straightforward but less-investigated feature of tolerance response is the time taken for the response. Delay of phenology is common in attacked and tolerating plants (Tiffin 2000). Phenological shifts, such as delays in germination, growth, and flowering, can be costly, as the life history strategies of plants aim for sophisticated temporal regulation of phase transition, which is critical for maintaining niches over generations (Preston and Fjellheim 2020). Although phenological shifts can be adaptive in the context of avoiding herbivory (Lucas-Barbosa et al. 2013), suboptimal regulation of the phase transition may result in a dramatic loss of plant fitness (Scheepens and Stöcklin 2013). While phase transitions are controlled by external and internal cues in some plants (e.g., seed dormancy break by karrikin [Flematti et al. 2004] and vernalization-induced flowering [Chouard 1960]), other plants, including many ruderal species, show less controlled phase transitions (Montesinos 2022). Spontaneous phase transitions in such species (e.g., *Erigeron annuus* and rapid-cycling accessions of *Arabidopsis thaliana*) can cause variations in the recruitment timing in newly disturbed areas. This variation often plays an important role in the competition (Boeken 2018). Thus, the impact of the tolerance response on plant fitness must encounter the effect of altered phenology on offspring production.

Here, we tested the hypothesis that the delayed phenology in offspring of tolerating plants is costly using a biennial herb *E. annuus* L. (Asteraceae) and its stem-boring herbivore *Phytoecia rufiventris* Gautier (Cerambycidae; Laminae). As a ruderal species, *E. annuus* produces a lot of seeds without dormancy with high dispersal rate. Despite their long dispersal distance, the limited niche of their germination might result in intraspecific competition in this species (Stratton 1992a, 1995). *Phytoecia rufiventris* females girdle the stem of *E. annuus* before endophytic oviposition to facilitate larval growth (Choi et al. 2024). The upper part of the girdled plants including the shoot apical meristem dies which is followed by emergence of the axillary buds at the lower part.

We first examined whether the tolerance response of *E. annuus* fully compensates for the loss of the upper part by the girdling behavior and damage by larval attack. Then we measured the competition-mediated cost of delayed phenology in the offspring of tolerated plants. Finally, we tested whether excluding competition and herbivore affect the phenology-associated cost to collectively show that the ecological consequence of tolerance response largely depends on the offspring's competition.

Materials and Methods

Plant growth

Erigeron annuus seeds were collected in Daejeon, South Korea in 2019. The *E. annuus* plants were grown following previously described methods (Choi et al. 2024). Third-generation of field-collected seeds were used in this study. The seeds were germinated on a germination media plate (Gamborg's B5 [Duchefa, Haarlem, Netherlands] 3.16 g/L, plant agar [Duchefa] 6 g/L, pH 6.8) and transplanted into the high soil (Bunong, Gyeongju, Korea) 12 days later. No additional fertilization was applied. Plants were grown under long-day conditions (16L:8D) at 26°C. We used 8-week-old plants for the experimental girdling.

Experimental girdling and egg inoculation

Experimental girdling and egg inoculation were performed as previously described with modifications (Choi et al. 2024; Lee et al. 2016). Briefly, the vascular bundles of *E. annuus* were cut into two lines using Westcott scissors, and an egg was inoculated between the two girdles. In the non-girdling group, egg inoculation was performed without girdling. In groups with egg inoculation, larval feeding was confirmed. The number of flowers was counted 3 weeks after girdling.

Field monitoring

The tolerance responses of *E. annuus* plants attacked by *P. rufiventris* were monitored in a field in Cheongju, South Korea (36°37’31.2”N, 127°27’11.4”E). Freshly girdled *E. annuus* plants and adjacent non-girdled *E. annuus* plants were labeled (n = 11–15) on the 15th and 17th, May 2020. The chlorophyll content of the 10th true leaf was measured using a SPAD meter (SPAD-502Plus; Konica Minolta, Tokyo, Japan) during the first two weeks of observation. SPAD values indicated the level of chlorophyll in the leaf samples. The numbers of branches longer than 15 cm and flowers were counted weekly until the monitored plants were senesced. A total of 30 flowers were randomly collected for diameter measurement on 17th July 2020 (9 weeks post-girdling).

Greenhouse competition experiment

Two *E. annuus* plants (early germination [EG] and delayed germination [DG]) that were germinated at intervals of 2 weeks were transplanted into pots (9 × 9 × 9 cm) 6 cm apart at greenhouse (16L:8D, 26°C) with 150 W light (PAR; 200–300 μmol m^{-2} s^{-1}). The 2 weeks of gap was determined according to the observed delay in flower production by the girdling behavior (Fig. 1D). The rosette diameter, number of branches, total branch length, and number of flowers were measured every week for 6 weeks. Six weeks after transplantation, the aboveground parts of both plants were sampled for fresh weight measurements. The sampled plants were dried in a 60°C dryer for 7 days to measure the dry weight.

Field competition experiment

The EG and DG *E. annuus* plants were germinated at 2-week intervals and transplanted individually into high soil (Bunong) pots (9 × 9 × 9 cm). Six weeks after the germination of EG plants, both EG and DG plants were transplanted to the field spot (Cheongju, Korea; 36°37’31.3”N, 127°27’14.8”E) in alternating order, 7 cm apart. Each pair

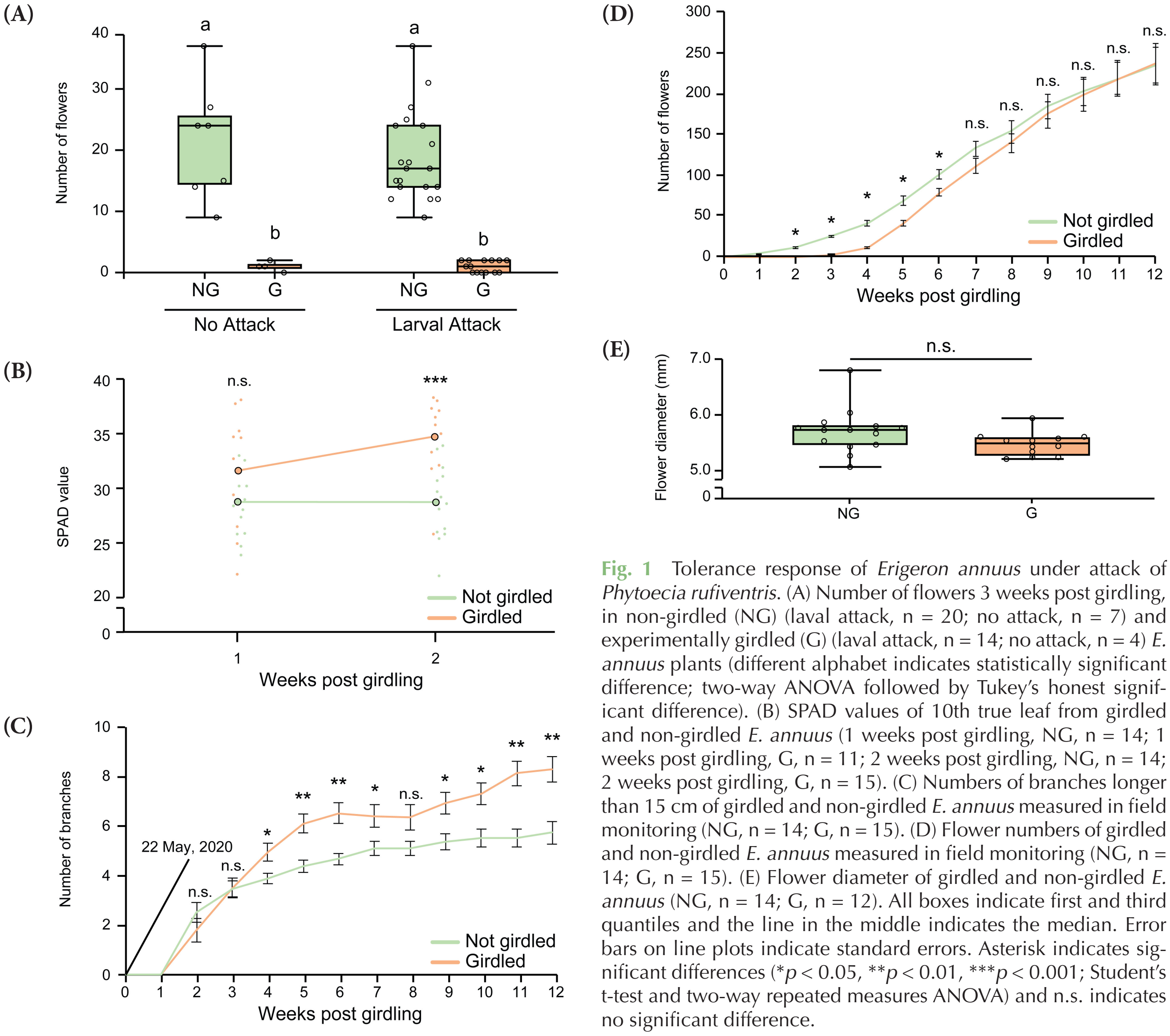

Fig. 1 Tolerance response of *Erigeron annuus* under attack of *Phytoecia rufiventris*. (A) Number of flowers 3 weeks post girdling, in non-girdled (NG) (laval attack, n = 20; no attack, n = 7) and experimentally girdled (G) (laval attack, n = 14; no attack, n = 4) *E. annuus* plants (different alphabet indicates statistically significant difference; two-way ANOVA followed by Tukey’s honest significant difference). (B) SPAD values of 10th true leaf from girdled and non-girdled *E. annuus* (1 weeks post girdling, NG, n = 14; 1 weeks post girdling, G, n = 11; 2 weeks post girdling, NG, n = 14; 2 weeks post girdling, G, n = 15). (C) Numbers of branches longer than 15 cm of girdled and non-girdled *E. annuus* measured in field monitoring (NG, n = 14; G, n = 15). (D) Flower numbers of girdled and non-girdled *E. annuus* measured in field monitoring (NG, n = 14; G, n = 15). (E) Flower diameter of girdled and non-girdled *E. annuus* (NG, n = 14; G, n = 12). All boxes indicate first and third quantiles and the line in the middle indicates the median. Error bars on line plots indicate standard errors. Asterisk indicates significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Student’s t-test and two-way repeated measures ANOVA) and n.s. indicates no significant difference.

of EG and DG plants were planted 30 cm apart from another pair (Fig. 2A). To mimic the overwintering of natural *E. annuus* plants, transplanting was conducted in October 2020, and the plants were monitored until July 2021. Leaf herbivory of rosettes was screened by visually measuring the damaged portion of the total rosette. The rosette diameter was measured weekly from October 2020 to April 2021, when active flowering was started. The number of flowering plants was counted weekly from January 2021 until the flowering of the EG and DG plants was saturated. The total lengths of the branches were measured weekly from April to June 2021. The number of flowers was mea-

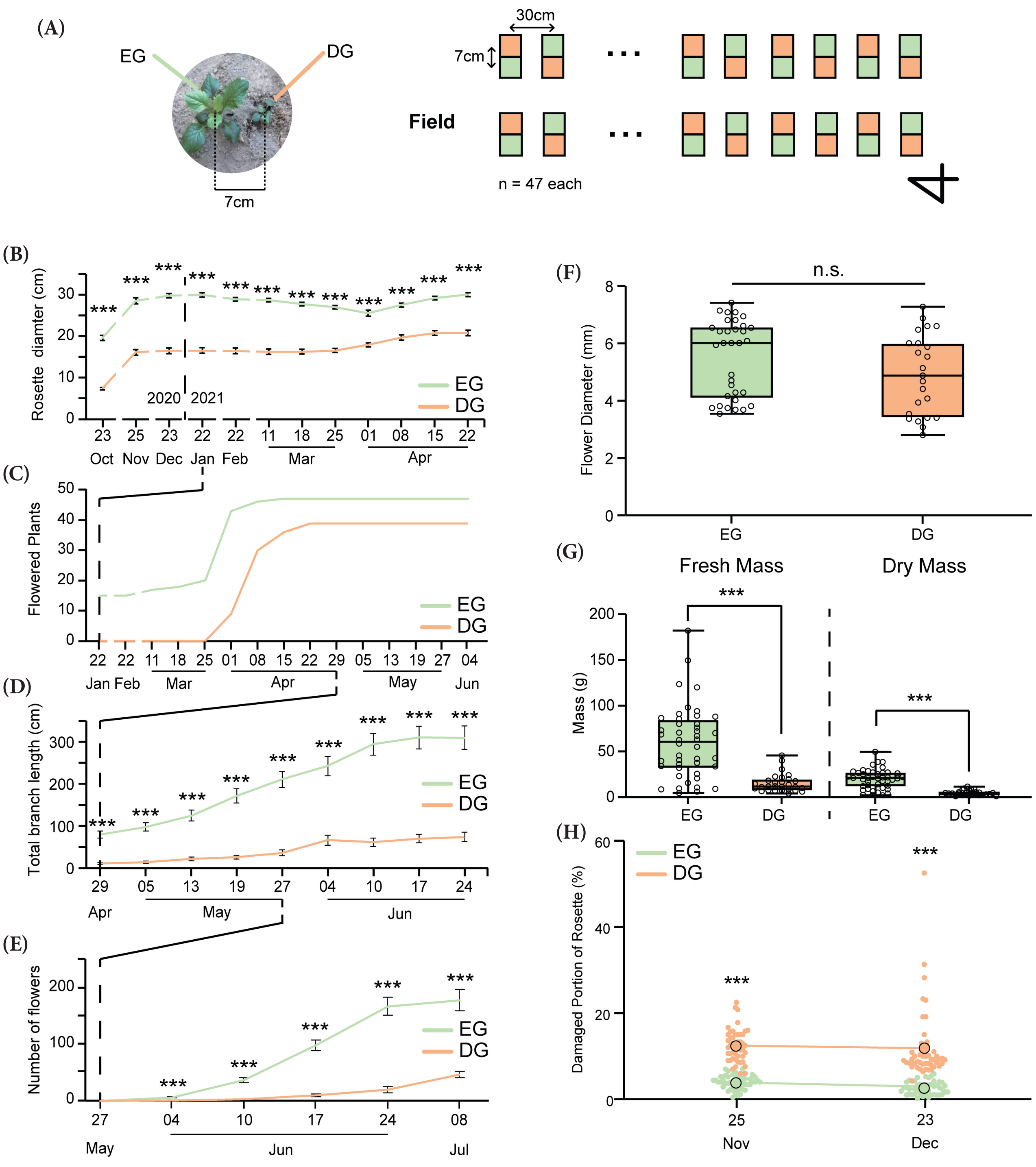

Fig. 2 Field competition assay using 2 week-DG. (A) Experimental setup of competition assay (EG, n = 47; DG, n = 47). (B-E) Time-course measurements on growth and reproduction-related parameters of EG and DG plants. Error bars indicate standard errors. (B) Rosette diameter, (C) number of bolted plants, (D) total length of branches, and (E) number of flowers are measured (two-way repeated measures ANOVA). (F) Diameter of flower from EG and DG plants (Student's t-test). (G) Fresh and dry mass of EG and DG plants at the end of the assay (Student's t-test). (H) Herbivore damage on EG and DG plants measured before overwintering season (Student's t-test). Error bars on line plots indicate standard errors. Asterisk indicates significant differences (***$p < 0.001$; Student's t-test and two-way repeated measures ANOVA) and n.s. indicates no significant difference. EG: early germination; DG, delayed germination.

sured weekly from May to July 2021 when flower production was active. Thirty flowers were randomly chosen to measure the floral diameter. At the end of the flowering season (July), the above-ground parts of both plants were sampled and used for fresh and dry weight measurements.

Competition and herbivory exclusion field experiment

The EG and DG plants were transplanted as in the field competition experiment at Jeungpyeong-gun, South Korea (36°44’32.63”N, 127°36’48.41”E) in October 2021, 15 cm apart to exclude competition. The transplantation site (4 × 4 m^2) was covered with a mesh (warp: 0.6 mm, weft: 0.2 mm; KwangSin Fabrics, Daegu, Korea) to exclude aboveground herbivory. The rosette diameter was measured every week from March to April 2022. The number of branches and the total branch length were measured every week from April to June 2022. The number of flowers was counted every week from May to June 2022.

Statistical analyses

The unpaired Student’s t-test was used to compare continuous variables between the two groups. Two-way repeated measure ANOVA was used for analysis with time-course data then followed by Tukey’s honest significant difference test as a post hoc analysis. All statistical tests were conducted using R (version 4.2.1; http://www.r-project.org/).

Results

Tolerance response of *E. annuus* against the attack of girdling beetle *P. rufiventris*

We characterized the life history of *E. annuus* with and without the attack of *P. rufiventris* (Fig. 3). Overwintered *E. annuus* began to bolt in April and produced flowers in May. The seeds germinated and overwintered at the rosette stage. *Phytoecia rufiventris* laid an egg inside the stem of *E. annuus* after girdling the stem from April to May. Girdling behavior killed the upper part, stimulating axillary bud emergence. Seed production in girdled *E. annuus* was therefore delayed compared with that in non-girdled *E. annuus* plants.

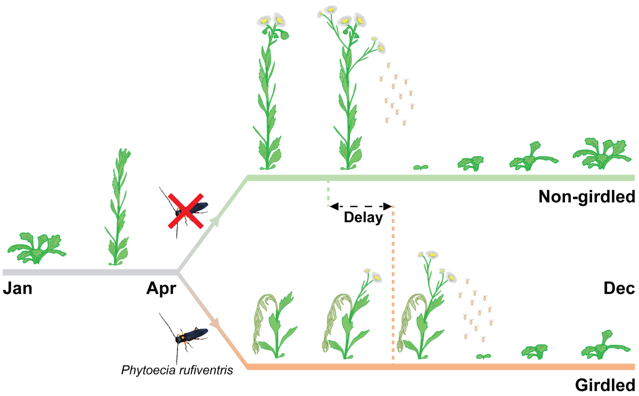

Fig. 3 Life history of *Erigeron annuus* and effect of *Phytoecia rufiventris*. *Erigeron annuus* flowers once a year and overwinters as rosette. Girdling of *P. rufiventris* delays seed production by killing the shoot apical meristem.

To assess the fitness loss caused by girdling beetles, we measured the number of flowers in experimentally girdled and non-girdled *E. annuus* plants with and without larval attack. Three weeks after girdling and egg inoculation, girdled *E. annuus* produced significantly fewer flowers than the non-girdled *E. annuus* (Fig. 1A). Flower production was not affected by larval feeding inside the stems.

We then measured the long-term effects of girdling on *E. annuus* growth and reproduction of naturally girdled *E. annuus* in the field. Two weeks after girdling, girdled *E. annuus* plants had higher chlorophyll content than non-girdled plants (Fig. 1B). Subsequently, the axillary bud emergence was boosted in girdled *E. annuus,* and the number of branches exceeded that in non-girdled *E. annuus* 4 weeks after girdling (Fig. 1C and Table S1). The extra branches of girdled *E. annuus* persisted throughout the flowering season of *E. annuus*. The higher number of axillary branches in girdled *E. annuus* led to flower production restoration. Initially, girdled *E. annuus* plants produced significantly fewer flowers than non-girdled plants. However, the difference diminished over time and lost significance seven weeks after girdling (Fig. 1D and Table S1). Consequently, the difference in the number of flowers in girdled and non-girdled *E. annuus* was not significant by 12 weeks after girdling when the flowering season of *E. annuus* was complete. We further compared flower sizes to assess the quality of each flower. The flower size of girdled and non-girdled *E. annuus* did not differ significantly (Fig. 1E). Thus, we concluded that the loss of shoot apical meristems in girdled *E. annuus* restores reproductive production by boosting growth at the end of the life cycle.

Delayed germination cause fitness costs in offspring under intraspecific competition

While the number of flowers produced by girdled and non-girdled *E. annuus* was similar at the end of their lifetime, flower development in girdled *E. annuus* was approximately 2 weeks slower than that in non-girdled *E. annuus* in the field (Fig. 1D). As most *E. annuus* seeds do not undergo dormancy, delays in the initial floral production are likely to cause delays in early-period offspring germination in newly disturbed areas. Therefore, we sought to determine the consequences of DG in the context of intraspecific competition using a greenhouse competition experiment. One *E. annuus* plant germinated (EG) in a pot, and another *E. annuus* plant germinated (DG) at an adjacent site in the same pot 2 weeks later (Fig. 4A). Competition

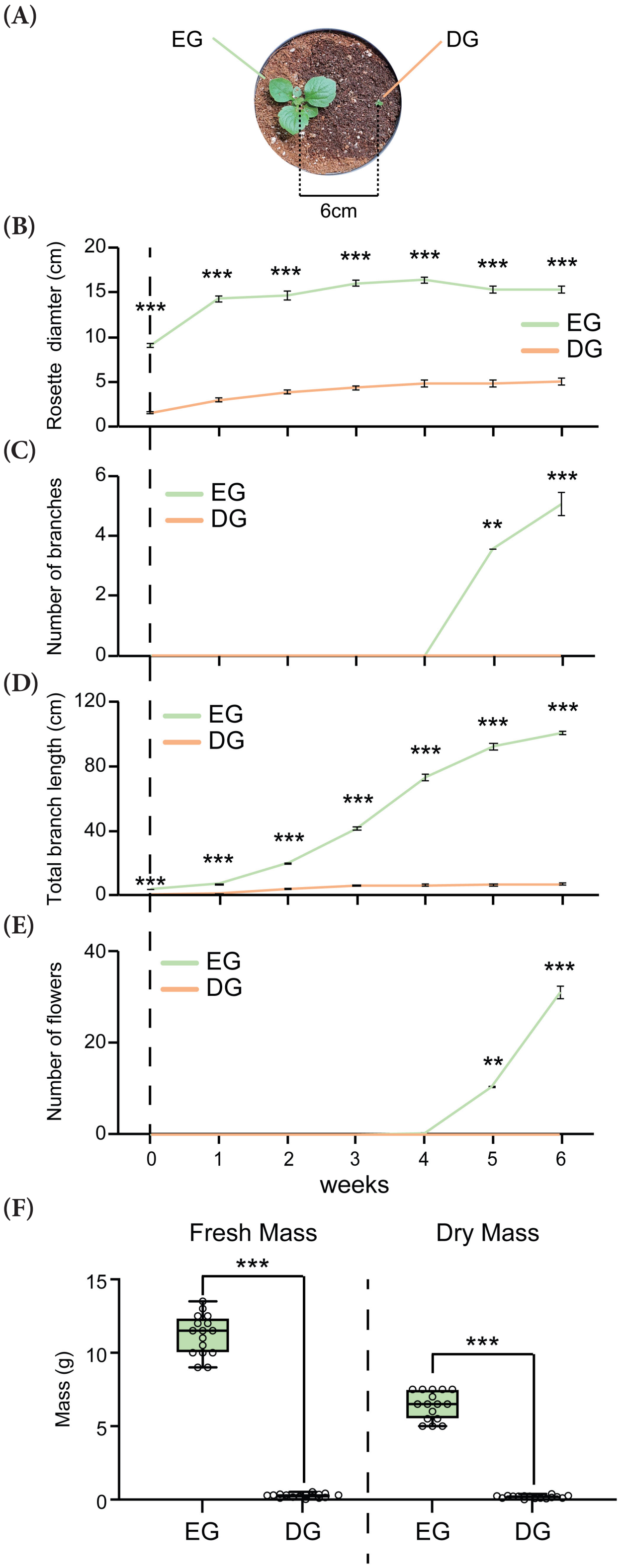

Fig. 4 Greenhouse competition assay using 2 week-delayed germination. (A) Experimental setup of competition assay (EG, n = 16; DG, n = 16). (B-E) Time-course measurements on growth and reproduction-related parameters of EG and DG plants (**$p < 0.01$, ***$p < 0.001$; n.s., no significant difference; Student's t-test and two-way repeated measures ANOVA). (B) Rosette diameter, (C) number of branches, (D) total length of branches, and (E) number of flowers are measured. (F) Fresh and dry mass of EG and DG plants at the end of the assay (Student's t-test). Error bars on line plots indicate standard errors. EG: early germination; DG, delayed germination.

between the EG and DG plants was strong enough to inhibit the normal growth of DG compared to that in EG plants. The rosette sizes of the EG plants were larger than those of the DG plants (Fig. 4B and Table S2), indicating a higher growth potential in many biennial plants, including *E. annuus*. Indeed, the DG plants produced no axillary branches, whereas the EG plants produced approximately five branches throughout the observation period (Fig. 4C, D, and Table S2). Importantly, the supremacy of growth in EG plants led to a larger number of flowers than that in DG plants, denoting the fitness cost of DG (Fig. 4E and Table S2). Poor growth of DG plants compared to that of EG plants was also observed in total biomass measurements (Fig. 4F).

Several variables, such as winter dormancy, changing day length, and biotic stresses, were not reflected in the greenhouse experiment. To account for naturally occurring conditions, we further conducted a field competition experiment. *Erigeron annuus* plants in the vegetative phase were transplanted to the field with a gap of 2 weeks in germination (EG and DG) at a close distance in which the canopies of EG and DG plants overlapped (Fig. 2A). We monitored the plants to track their growth and flower production during intraspecific competition. While the overwinter survival of two groups was not significantly different (EG: 93.6%, DG: 97.8%, n = 47), the initial gap in rosette size between the EG and DG plants persisted throughout winter and subsequently extended to the spring season (Fig. 2B and Table S3). This gap implies a difference in the resources used for growth, which was represented by the number of successfully flowered plants in the subsequent spring season (Fig. 2C). The DG plants bolted less than the EG plants and produced fewer branches than the EG plants (Fig. 2D and Table S3). These defects in growth in DG plants resulted in a significantly lower number of flowers (Fig. 2E and Table S3) than in EG plants at the end of the flowering season of *E. annuus*. The number of flowers produced by DG plants was not only smaller than the flower number of EG plants at the corresponding time points but also smaller than that at 2 weeks before (the corresponding stage). As shown in similar flower size of DG and EG plants, the quality of flower is assumed to be similar in two groups (Fig. 2F). The total biomass of the DG plants was also lower than that of the EG plants (Fig. 2G). Interestingly, DG plants were more susceptible to herbivore attacks than EG plants before overwintering (Fig. 2H), implying an additional cost of DG under natural conditions. Collectively, intraspecific competition caused serious cost in DG plants in natural environments as well as in the controlled greenhouse experiment.

The fitness cost involved in germination delay depends on biotic interactions

We lastly tested whether the significant fitness cost of

DG persisted without intraspecific competition or herbivory. To avoid competition, *E. annuus* plants were transplanted to the field with 2 weeks of the gap in germination (EG and DG) but without overlap in their canopies. The field plot was covered with a mesh to exclude natural herbivores (Fig. 5A). Thereafter, we measured the growth and reproduction of EG and DG plants over consecutive year. Interestingly, EG and DG plants did not produce significantly different rosette sizes after overwintering without competition (Fig. 5B and Table S4). Moreover, DG plants produced more axillary branches than EG plants (Fig. 5C, D, and Table S4). As in the aforementioned experiments, more axillary branches in the DG plants resulted in greater flower production than in EG plants (Fig. 5E and Table S4). Collectively, DG plants outperformed EG plants in environments without competition or herbivory.

Discussion

In comparison to the broad evidence supporting the benefits of tolerance responses of plants under attack, what factors hinder fitness compensation by tolerance response are unclear. Measuring fitness using appropriate parameters is crucial for assessing the adaptive value of a trait in an ecologically relevant manner (Erb 2018). Traits or phenomena can affect plant fitness at various stages of the plant life cycle (Boege and Marquis 2005; Swope and Parker 2010). Although plant growth sufficiently represents fitness in some cases, the production and/or germination of seeds is considered proxies closer to actual fitness. In this study, we showed that restoring the number of flowers did not fully compensate for the fitness loss caused by girdling beetle attacks, under intraspecific competition in offspring generation.

Plants under herbivore attack actively increase their photosynthetic performance to compensate for damage (Halitschke et al. 2011; Retuerto et al. 2004). *Erigeron annuus* also showed increased chlorophyll content, which could represent enhanced photosynthetic performance after girdling by *P. rufiventris*. Moreover, insect attacks enhance branching, which results in increased reproductive organ production (Paige and Whitham 1987). Although girdling behavior caused a serious loss in flower number due to the loss of shoot apical meristem in the short term, increased axillary branch emergence in girdled *E. annuus* restored the number of flowers within 7 weeks of girdling. At the end of the flowering season, girdled *E. annuus* produced not significantly different numbers or sizes of flowers compared to those in non-girdled *E. annuus*.

Our attention was drawn to the 2 weeks of delay in flower production in girdled *E. annuus* plants. Plant-insect interactions often result in phenological shifts in plants, and the scale of the shift varies from hours (Kessler et al. 2010)

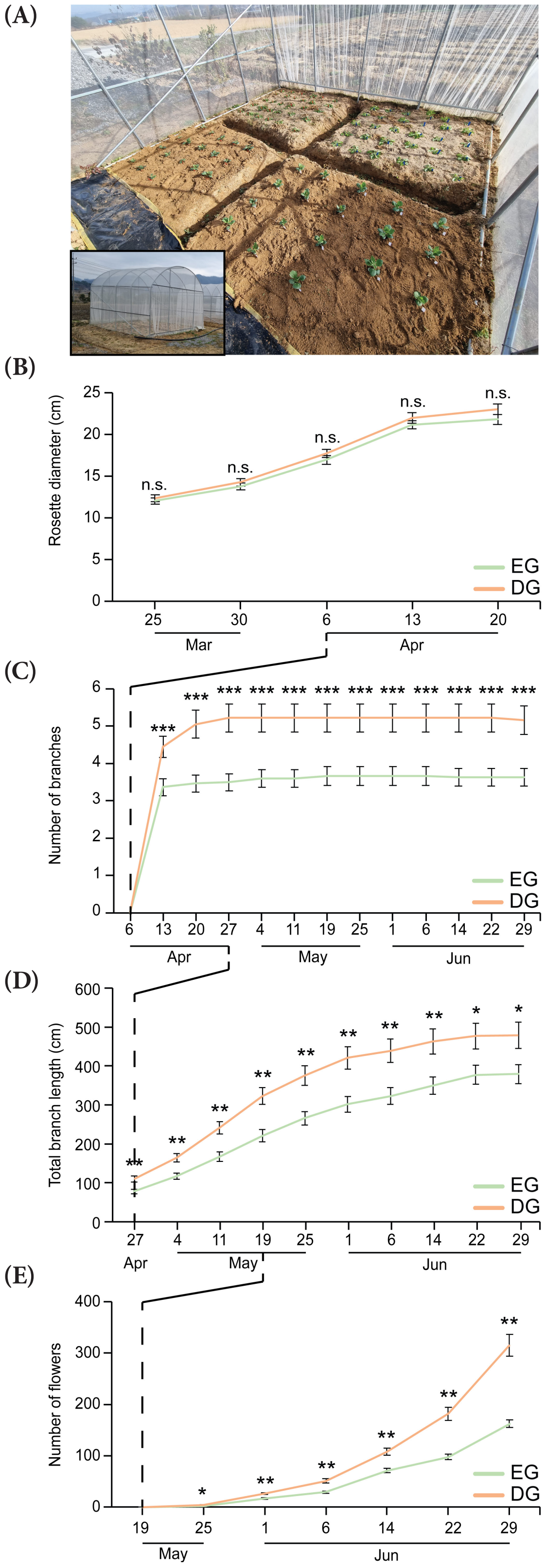

Fig. 5 Field non-competition growth assay with 2 weeks-delayed germination. Time-course measurements on growth and reproduction-related parameters of EG and DG plants are conducted. Error bars on line plots indicate standard errors. (A) Field site under herbivory exclusion and non-competition, (B) Rosette diameter, (C) number of axillary branches, (D) total length of branches, and (E) number of flowers were measured. Asterisk indicates significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Student's t-test and two-way repeated measures ANOVA) and n.s. indicates no significant difference. EG: early germination; DG, delayed germination.

to months (Sercu et al. 2020) in the context of defense. The phenological shift is trans-generationally transmitted to make a 'mismatch' with herbivory (Kessler et al. 2010; Lucas-Barbosa et al. 2013; Sercu et al. 2020). However, the delay in flower production in girdled *E. annuus*, which is likely to persist through seed germination due to lack of seed dormancy, caused a fitness cost in the intraspecific competition of offspring rather than benefits from the mismatch. The DG plants, which were referred to be produced by girdled *E. annuus* plants, showed smaller biomass and produced fewer flowers than did the EG plants, which were referred to be produced by non-girdled *E. annuus* plants.

The consequences of competition between DG and EG plants were similar in the greenhouse and field experiments. Our field competition experiment was designed to test whether the competition-associated cost of delay in germination is also exhibited in the field, which has different conditions from those of greenhouse experiments (Forero et al. 2019; Heinze et al. 2020). For instance, rosettes tolerate cold stress during winter and transition into a reproductive form in consecutive springs using accumulated resources (Friedman 2020; Ramachandran et al. 2023). Moreover, herbivore damage was more severe in DG plants than in EG plants during the rosette stage. As plant ontogeny significantly affects the degree of defense and the early stages are particularly vulnerable to herbivory (Fenner et al. 1999), it can be suspected that the growth defect by the intraspecific competition caused weaker defense in DG plants.

Notably, the growth and reproductive trends of the EG and DG plants were opposite in the two different field experiments performed with and without biotic interactions. Excluding intraspecific competition and herbivory led to more flowers than EG plants in the following year. The observed data may have resulted from artifacts originated from discrepancies between plant developmental stages and environmental conditions. The mismatch between resource demand determined by plant ontogeny and resource availability in the environment could cause fitness costs (Nakazawa and Doi 2012). Therefore, the ontogeny of plants significantly affects the recruitment of plants at the community level (Ramachandran et al. 2023). Moreover, our data might also show an aspect of temporal variation in the fitness effect of germination timing. The effect of germination timing on the lifetime fitness of plants varies over years in the field experiments (Postma and Ågren 2018). As our competition assay and non-competition assay were conducted in different year, the direct comparison between two could be misinterpreted.

Ruderal species, including *E. annuus*, adopt an opportunistic strategy of occupying an empty niche caused by disturbance (Montesinos 2022). Disturbances occur in various ways in ecosystems, and their timing is unpredictable. Therefore, the long seed dispersal period of pioneer species might be adapted to increase the chance of invading disturbed populations (Walker 2011). The lack of seed dormancy in *E. annuus* (Hayashi and Numata 1967) indicates that there is a long seed germination period following the long seed dispersal period for a growing season. Because dispersed seeds of *E. annuus* inhabit under various levels of competition, the girdling-induced delay in flower production might increase cost of losing access to the disturbed population and falling short of preparing (Bennington and Stratton 1998; Hayashi 1984; Hayashi and Numata 1967). Plant-herbivore interaction exerts selection on both organisms and the effects are transmitted over a long period (Agrawal and Maron 2022). Although our study was conducted under the assumption that tolerance-associated delay in flower production affects intraspecific competition and herbivory in the subsequent generation, evolutionary changes in plants and herbivore rooting for such phenomena needs to be studied in the future. Moreover, interspecific competition which was not considered in the current study but might strongly affect the fitness of tolerating plants, deserves further investigation.

Our data emphasize that the restoration of floral production in plants under herbivory does not fully compensate for fitness in the context of tolerance responses. However, the current results do not reflect all aspects of plant fitness. Although girdled *E. annuus* plants started to produce flowers 2 weeks later than non-girdled plants, flower and seed production continued longer than the period (Stratton 1992b). Many ruderal plants tend to colonize newly disturbed areas (Montesinos 2022). A broad flowering period is also another characteristic of *E. annuus* (Stratton 1992b). Due to this, the negative effect of delayed flowering due to girdling may not affect the entire life cycle, but may only affect newly disturbed areas that occur early in flowering. The seeds produced in the later period of the reproductive phase of *E. annuus* could overcome the fitness cost that suffered seeds produced by girdled plants. As recruitment time influences plant fitness (Boeken 2018), additional research is necessary to consider the establishment time in natural habitats and flowering time delay caused by herbivory. For instance, seeds produced in the early period would strongly benefit from the gap of germination while seeds from the later period would benefit only in marginal degree.

Conclusions

In this study, we describe an overlooked fitness cost that occurs during tolerance responses to insect attacks. Although the tolerance response enabled attacked plants to restore the number of flowers, the restoration caused 2 weeks of delay in the germination of offspring. DG was found to cause critical defects in offspring under intraspecific competition. In other words, the fitness loss of being attacked by girdling behavior is inflicted on the offspring produced by girdled plants, even with a tolerance response. We conclude that reproductive tolerance which is considered to result in similar fitness between damaged and undamaged plants, actually may not fully compensate for the fitness depending on competition and herbivory. Furthermore, we suggest future research on plant defense, especially those involving phenological shifts, to take into account the effect of the shift in offspring to more accurately measure the adaptive value of plant defense traits.

Supplementary Information

Supplementary information accompanies this paper at https://doi.org/10.5141/jee.24.048.

Table S1. Two-way repeated measures ANOVA results from observations on girdled and non-girdled plants. **Table S2.** Two-way repeated measures ANOVA results from observations on lab competition assay. **Table S3.** Two-way repeated measures ANOVA results from observations on field competition assay. **Table S4.** Two-way repeated measures ANOVA results from observations on field non-competition assay.

Abbreviations

EG: Early germination
DG: Delayed germination

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Authors' contributions

JMK, MSC, and YJ designed the study. JMK, MSC, JL, and YJ performed the experiments. JMK and MSC analyzed the data. JMK, MSC, and YJ wrote the manuscript.

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Availability of data and materials

The raw data deposited at Dryad (https://datadryad.org/stash/share/cJ5W5Z5CWS54AbXwJgexTqX4Dt0pD7CQaBdSvpVDCJs).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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