A technique for transplanting gall-making insects: impacts on gall-maker and parasitoid larvae

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Abstract—Past studies of gall-maker–host interactions have been hampered by an inability to conduct experimental transplants of individuals between host plants. We describe a method for transplanting gall-maker larvae between galls on different individual host plants. Our method involves removing and inserting larvae through slits cut in young galls, and allows for healing and continuing growth of the gall. We developed and tested our method with larvae of the gall-making moth *Gnorimoschema gallaesolidaginis* Riley (Lepidoptera: Gelechiidae) on its two host plants, *Solidago altissima* L. and *S. gigantea* Ait. (Asteraceae). For three of four host × year combinations, unparasitized larvae survived at similar rates in transplants and controls. On one host in one year, transplant survival was low, possibly as a result of severe drought stress. Interestingly, survival of parasitized gall-maker larvae was lower in transplants for three of four host × year combinations, suggesting that gall-makers stressed by parasitoid attack are less able to tolerate transplant stress. Our technique may be applicable to many other gall-maker species, especially those making relatively thin-walled galls, and should represent a valuable new tool for the study of gall-maker–host interactions.

Résumé—Les études antérieures des interactions entre les insectes galligènes et leur hôtes ont été entravées par l'impossibilité de transplanter expérimentalement les individus entre les plantes hôtes. Nous décrivons une méthode pour transplanter les larves galligènes d'une galle à une autre sur différentes plantes hôtes individuelles. Notre méthode consiste dans le retrait ou l'insertion des larves par une fente pratiquée sur de jeunes galles et elle permet la cicatrisation et la poursuite de la croissance de la galle. Nous avons mis au point et testé notre méthode avec les larves du papillon de nuit galligène *Gnorimoschema gallaesolidaginis* (Riley) (Lepidoptera: Gelechiidae) sur ses deux plantes hôtes, *Solidago altissima* L. et *S. gigantea* Ait. (Asteraceae). Dans trois des quatre combinaisons hôte × année, les larves non parasitées dans les expériences de transplantation ont eu des taux de survie semblables à ceux des témoins. Dans une des années, chez un des hôtes, la survie après la transplantation était basse, possiblement à cause d'un stress dû à une forte sécheresse. Il est intéressant de noter que la survie des larves galligènes parasitées a été plus faible dans 3 des 4 combinaisons hôte × année, ce qui laisse croire que les insectes galligènes sous le stress d'une attaque de parasitoïdes sont moins capables de tolérer le stress de la transplantation. Notre technique peut vraisemblablement s'appliquer à plusieurs autres espèces d'insectes galligènes, particulièrement à celles qui construisent des galles à parois relativement minces; elle représente un nouvel outil précieux pour étudier les interactions galligène-hôte.

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Introduction

Phytophagous insects account for a large fraction of animal diversity on Earth and are both economically and ecologically important. Experimental techniques for assessing effects of insects on plant hosts and of host plants on insects are critical to the understanding of plant–

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insect interactions, and an important class of techniques involves the transfer of insects between individual plants (of the same or different species). Such transfers are central to the assessment of tolerance and resistance of plants, induction of defence, fitness impacts of herbivory, and causes and consequences of host specialization (*e.g.*, Van Zandt and Agrawal 2004; Bird and Hodkinson 2005; Lau 2006).

Although manipulation of free-living insect herbivores is usually straightforward, transplantation of internally feeding herbivores (leaf miners and gall-makers) is much more difficult. Leafminers have been successfully tranplanted into artificially created mines (Gratton and Welter 1999), but for gall-makers, the only technique available has been caging of adult females before oviposition (*e.g.*, Egan and Ott 2007). This is unfortunate because gall-makers include many interesting herbivore radiations with very tight physiological interactions between insect and host (Cook *et al.* 2002; Stone and Schonrogge 2003) — and, likely as a consequence, with very high host specificity (Nyman *et al.* 2000; Cook *et al.* 2002). Conspicuously diverse gall-maker radiations are found in many insect clades, including the cecidomyiid flies (Diptera: Cecidomyiidae; Gagné 1989; Joy and Crespi 2007), sawflies (Hymenoptera: "Symphyta"; Nyman *et al.* 2000), and cynipid wasps (Hymenoptera: Cynipidae; Cook *et al.* 2002; Stone *et al.* 2002), and such radiations may provide good examples of diversification *via* host-race formation and ecological speciation (Stireman *et al.* 2005). In many studies of gall-maker ecology and evolution, questions are asked about plant traits that affect gall-maker preference and performance (*e.g.*, Cronin and Abrahamson 2001; Yamazaki and Ohsaki 2006), plant defences (*e.g.*, Fernandes and Negreiros 2001), trade-offs in adaptation to different host plants (*e.g.*, Cronin and Abrahamson 2001; Seehawer 2002), and effects of host-shifting on parasitoid attack (Heard *et al.* 2006) — all areas where rigorous hypothesis-testing is difficult if one cannot manipulate the insect. Furthermore, if manipulations are limited to the oviposition stage, it is difficult to separate larval performance from host preference or to identify mechanisms of herbivore resistance.

Transplanting gall-makers between hosts following gall initiation poses major challenges because inserting/removing gall-maker larvae into/from galls inevitably damages the gall and the host plant. To date, no study has, to our knowledge, demonstrated a method for transplanting gall-makers between galls on different host individuals or species. In this study we describe and test such a method for transplanting the goldenrod elliptical-gall moth, *Gnorimoschema gallaesolidaginis* Riley (Lepidoptera: Gelechiidae), on its two hosts, *Solidago gigantea* Ait. and *S. altissima* L. (Asteraceae) (giant and late goldenrod, respectively). Our test includes consideration of how other stresses (drought and parasitoid infection) influence larval tolerance of transplantation. Our method may be applicable to many other gall-makers, particularly those that make relatively thin-walled or large-chambered galls.

Materials and methods

Study organisms and study sites

Solidago gigantea and *S. altissima* are clonal perennials, regrowing each spring from underground rhizomes as well as recruiting from seed. These ruderals are distributed over much of temperate North America in grasslands and in disturbed habitats such as abandoned fields, roadsides, and other areas of secondary succession. Their habitats also overlap in much of their range (Semple and Cook 2006), and the two species are often densely intermixed in local populations.

Gnorimoschema gallaesolidaginis is a gall-making moth that attacks *S. altissima* and *S. gigantea* (and, rarely, their close relative *S. canadensis*). Subpopulations on *S. altissima* and *S. gigantea* are genetically distinct and likely constitute either well-developed host races or young cryptic species (Nason *et al.* 2002; Stireman *et al.* 2005). Adults lay eggs in the fall, eggs overwinter and hatch in spring, and larvae search for newly emerged goldenrod shoots. After selecting a goldenrod ramet, a larva will feed its way through the terminal leaf bud into the stem and induce the formation of a gall. *Gnorimoschema gallaesolidaginis* galls are roughly elliptical, and mature galls have hardened walls 2–4 mm thick and a large hollow chamber (often approximately 1 cm in diameter and approximately 2 cm along the central axis) in the center. The frequency of host-choice mistakes at the ramet-selection stage is unknown, but survival of a larva to pupation on the "wrong" host is very rare (Nason *et al.* 2002). *Gnorimoschema gallaesolidaginis* is attacked by a number of hymenopteran parasitoids of

which the commonest is *Copidosoma gelechiae* Howard (Hymenopera: Encyrtidae).

We tested our transplant technique in a large mixed-species *Solidago* L. population at the northern end of Tommy Thompson Park, Toronto, Ontario, Canada (43°39′N, 79°19′W). The park is situated on the Leslie Street Spit, an artificial spit jutting out into Lake Ontario. Our field site dates from near the beginning of construction in the 1950s and is dominated by open fields of grasses (Poaceae), sedges (Cyperaceae), wild carrot (*Daucus* L., Apiaceae), willows (*Salix* L., Salicaceae), and goldenrods and asters (Asteraceae). Both *S. altissima* and *S. gigantea* are present in mixed and single-species stands of many tens of thousands of ramets. *Solidago* ramets were easily assigned to species but we could not assign them to individual clones because rhizome connections are short-lived and clones typically interdigitate and thus only molecular genetic methods can successfully identify members of a clone (Maddox *et al.* 1989; Abrahamson *et al.* 1991).

Transplant method

In May of 2004 and 2005 we ran several transects across our study area and marked all galled ramets touching the transect with metal tags. Ramets were then paired by species and, as closely as possible, by size of both plant and gall. Gall-maker transplants were made reciprocally between paired plants (reciprocal transplants and larvae removed from and re-inserted into the same gall do not differ in survival rate; Seehawer 2002). At this time of year, neither galls nor gall-maker larvae have reached their full size, but the galls are well established and easily identified. In 2004 we made 18 transplants between *S. gigantea* ramets and 19 between *S. altissima* ramets, and in 2005 we made 22 transplants on *S. gigantea* and 25 on *S. altissima*.

For each transplant, galls on two paired *Solidago* ramets were slit open using a razor blade, with each cut made horizontally across the top of the gall adjacent to the stem and continuing down approximately one-third of the length of the gall. Each cut produced a flap of gall tissue that could be gently bent back to reveal the gall-maker larva. The larva was removed with a pair of fine artist's paint brushes and transported directly to the other gall of the transplant pair in a 4 mL polypropylene vial (Kimble 58552–4, Vineland, New Jersey). The larva was allowed to slide from the vial into the receiving gall. We then sealed up the receiving gall using narrow (0.5 mm) strips of electrical tape, holding the cut edges together and winding the tape from top to bottom of the gall. Well-sealed galls continued to grow, and over a period of 1–2 weeks the cuts healed fully, after which the electrical tape was removed.

Assessing gall-maker survival

Following transplantation, we compared the survival rate of transplanted larvae with that of unmanipulated controls. Unmanipulated galls were identified in the same set of transects and were marked and visited similarly to transplant ramets. In 2004 we collected 257 unmanipulated galls from *S. gigantea* and 217 from *S. altissima*, and in 2005 we collected 208 and 134 unmanipulated galls, respectively. In each year we collected galls from transplant and unmanipulated plants during the last week in August, just before moth emergence. Galls were opened and the contents scored in three categories: surviving gall-maker larva or pupa, surviving parasitoid, and dead or missing gall-maker.

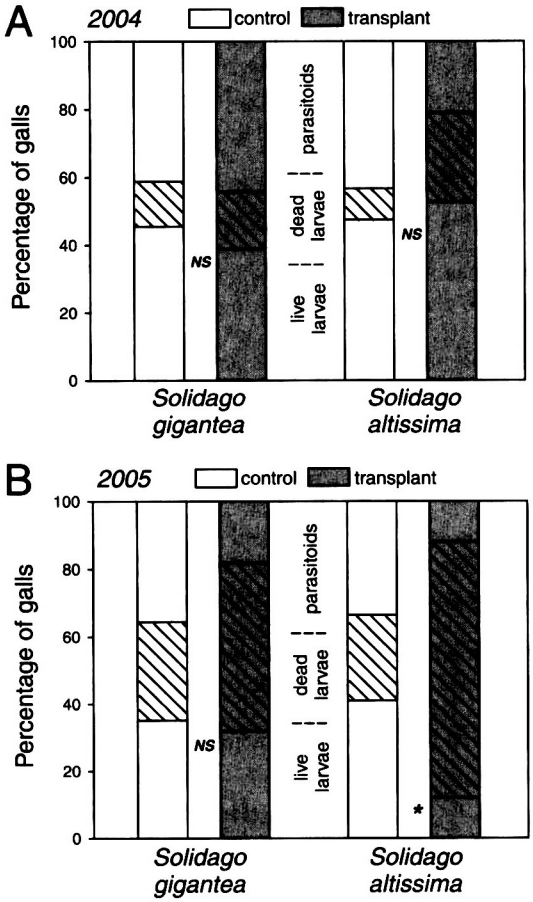
For each host species in each year we compared frequencies of occurrence of living gall-makers, living parasitoids, and dead gall-makers between transplant and unmanipulated galls using a 2×3 G test with Williams' correction (Sokal and Rohlf 1981). Significant heterogeneity in these frequencies could be generated by shifts in survival rates of either gall-makers or parasitoids, so we followed each significant test with 2×2 G tests comparing frequencies of occurrence of surviving gall-maker larvae (*vs.* parasitoids and dead larvae combined) between transplant and unmanipulated galls. The latter G tests are not independent of the 2×3 G tests motivating them, so statistical significance of individual tests should be interpreted with caution.

Results

In 2004 the fates of transplant and unmanipulated galls on *S. gigantea* did not differ (Fig. 1A; $G = 1.1$, 2 df, $P = 0.59$). On *S. altissima* the fates of transplant and unmanipulated galls did differ (Fig. 1A; $G = 35.5$, 2 df, $P < 0.0001$) because of fewer surviving parasitoids in transplant galls. Survival of gall-maker larvae did not differ between transplant and unmanipulated galls ($G = 0.19$, 1 df, $P = 0.66$).

In 2005 the fates of transplant and unmanipulated galls differed on both host-plant species (Fig. 1B; *S. gigantea*, $G = 36.0$, 2 df, $P < 0.0001$; *S. altissima*, $G = 21.0$, 2 df, $P =$

Fig. 1. Fates of the contents of unmanipulated galls (control) and gall-maker-transplant galls in 2004 (A) and 2005 (B) on two host-plant species, *Solidago gigantea* and *S. altissima* (NS, not significant; *, P = 0.014 (comparing comparisons are of lower bars ("live larvae" to the left and right of the significance symbols)). Sample sizes are as follows: in 2004, 18 transplants and 257 unmanipulated galls on *S. gigantea* and 19 transplants and 217 unmanipulated galls on *S. altissima*; in 2005, 22 transplants and 208 unmanipulated galls on *S. gigantea* and 25 transplants and 134 unmanipulated galls on *S. altissima*.



0.0003). For *S. gigantea* the difference could again be attributed entirely to a reduced frequency of surviving parasitoids in transplant galls (no difference in survival of gall-maker larvae; G = 0.09, 1 df, P = 0.76). For *S. altissima*, transplant galls contained fewer surviving parasitoids but also fewer surviving gall-maker larvae (G = 6.1, 1 df, P = 0.014).

Discussion

Transplant technique

In three of four host × year combinations, transplantation had no detectable effect on survival of unparasitized gall-maker larvae (Fig. 1). However, in 2005, survival of transplanted larvae in galls on *S. altissima* was poor. Unmanipulated *S. altissima* gall-makers also had lower survivorship in 2005 than in 2004 (G = 16.0, 1 df, P = 0.0006), although the drop in survival was much more severe for transplanted larvae. We suspect that this pattern is explained by severe drought stress on *S. altissima* plants in 2005. Total precipitation from May through July in that year (67 mm) was one-third of the average (206 mm) for 1971–2000 (data for Toronto Island Airport, 6 km from the study site); in contrast, 2004 was a wetter-than-average year, with 281 mm precipitation over the same time period (Environment Canada 2008). At our field site, *S. altissima* tends to occupy drier microhabitats than *S. gigantea*, and ungalled *S. altissima* (but not *S. gigantea*) ramets suffered very high mortality in 2005. Because surviving ramets were severely wilted or desiccated (G.H. Cox, personal observation), we believe that this mortality was the result of the obvious drought stress. Higher mortality of gall-makers on such ramets is not surprising, nor is the particularly high mortality of gall-makers exposed to the combined and possibly synergistic stresses of drought and transplantation.

At least for *G. gallaesolidaginis* we have shown that transplantation of gall-makers between galls on different host plants usually can be accomplished with little or no negative impact on survival. Although there were transplantation effects on survival for *S. altissima* in 2005, and are certainly possible for other gall-makers, most experimental designs can easily accommodate such effects with the inclusion of a transplantation control. No similar method has been available for any gall-maker (but for a leafminer transplant method see Gratton and Welter 1999).

We expect that our technique will be useful for studying a wide variety of galling insects — particularly many that make relatively thin-walled and large-chambered galls (because for such species opening a gall does not cause its complete destruction). Such thin-walled galls are found, for example, among sawflies (*e.g.*, *Pontania* Costa (Hymenoptera: Tenthredinidae) on willows), midges (*e.g.*, *Contarinia* Rondani

(Diptera: Cecidomyiidae) on chokecherry, *Prunus virginiana* L. (Rosaceae), and honeylocust, *Gleditsia triacanthos* L. (Fabaceae)), and gall-making Lepidoptera (*e.g.*, *Epiblema* Hübner (Tortricidae) and *Gnorimoschema* Busck on various hosts) (Fritz *et al.* 1996; Nyman *et al.* 2000; Stone and Schonrogge 2003). We suspect that further technical development will be required in order to successfully transplant gall-makers forming thicker walled galls, which represent an even more severe challenge for insect manipulations. For example, treatment with plant growth hormones during the gall-sealing stage might increase the rate of plant-tissue repair and permit transplantation of a wider range of gall-makers and hosts.

We carried out the transplants between size-paired ramets of the same host species, with the intent of focusing on potential mortality effects of the technique itself. However, future applications of the technique will likely involve transplants between host ramets varying in traits relevant to gall-maker–plant interactions (such as ramet size; Quiring *et al.* 2006) and between ramets of different host species. Interspecific transplants in particular should shed light on mechanisms of host specificity and host-race formation (Stireman *et al.* 2005). For example, it has sometimes been suggested that escape from natural enemies is an advantage that counters physiological maladaptation during the early stages of host shifting and host-race formation, but attempts to test this hypothesis (*e.g.*, Heard *et al.* 2006) have been handicapped by an inability to directly manipulate host affiliations of larvae. The addition of gall-maker-transplantation techniques to our arsenal of experimental tools should greatly enhance our understanding of plant–insect interactions.

Relative impacts of transplantation on parasitized and unparasitized gall-maker larvae

Although transplantation effects on gall-maker survival were detectable only for *S. altissima* in 2005, the overall fate of gall contents still differed between transplant and unmanipulated galls for both hosts in 2005 and for *S. altissima* in 2004. These effects arose largely because transplant galls yielded fewer surviving parasitoids (Fig. 1). For at least one major parasitoid (*Copidosoma gelechiae* Howard (Hymenoptera: Encyrtidae)), and likely for others, parasitoid oviposition occurred before transplantation, although parasitoid larvae are not visible inside the gall-maker until much later in the season. These results thus suggest that gall-maker larvae infected by parasitoids are less able to tolerate transplantation stress than are uninfected larvae. Sublethal effects of early parasitoid infection can compromise larval fitness (*e.g.*, Tien *et al.* 2001), perhaps as a result of costs associated with immune defence, and costs of defence can be contingent on the environment (Sandland and Minchella 2003).

Taken together, our data suggest that unparasitized larvae in galls on healthy plants tolerate transplantation stresses well, but that drought stress or parasitoid attack can act as a synergistic stressor that increases transplant mortality. Our work thus opens up new experimental avenues not only for the study of gall-maker–host interactions but perhaps for the study of gall-maker–parasitoid interactions as well.

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