

IMPORTANCE OF WOODY DEBRIS IN SEED GERMINATION OF *TIPULARIA DISCOLOR* (ORCHIDACEAE)¹

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Concerns about declining populations of terrestrial orchids make it important to identify the environmental factors crucial to seedling recruitment. This study shows that seedlings of *Tipularia discolor* (crane-fly orchid) primarily occur on decomposing wood. Extensive searches of decomposing logs and stumps in mature and successional forests revealed seedlings at 24 sites, of which 15 could be identified as originating from seven different deciduous trees and one conifer. Seeds were planted in natural habitats to test the hypothesis that germination requires decomposing wood. In one experiment, seeds were placed into soil at sites where adult plants were abundant; no germination resulted. In a second experiment, germination of seeds sown in ambient soil was compared with sowings in plots amended with decomposing wood collected from a stump where spontaneous seedlings grew. Germination was much more frequent in plots amended with decomposing wood. We conclude that germination of *T. discolor* is stimulated in substrates that contain decomposing wood; judging from the occurrence of spontaneous seedlings, wood from a variety of tree species offer a suitable substrate.

Key words: decomposing wood; forest ecosystem; germination; mycoheterotrophy; orchid mycorrhiza; seed biology; *Tipularia*; Orchidaceae.

Orchid populations generally produce vast amounts of seeds. Even with a low success rate of fruiting, the seeds produced by a single pollination often outnumber the individuals in the population. Hence the processes that determine germination and survival of seedlings have an overriding importance on the expansion or decline of populations (Rasmussen and Whigham, 1998).

Among the few long-lived woodland orchids that have been studied, seedling recruitment in *Cypripedium acaule* Aiton appears to be uncommon (Gill, 1996); few new plants appeared during the first 13 yr of observation. Only after disturbance of the tree canopy by fire and gypsy moth defoliation did large numbers of recruits appear within the marked population. Since it was impossible to determine whether the new plants were actual seedlings or plants reappearing after a sojourn belowground in a purely mycotrophic stage, we cannot know whether the perturbation of the environment affected seedling success, reversal to photosynthesis of established plants, or both. Recruitment also seemed extremely rare in populations of *Tipularia discolor* (Pursh) Nuttall (crane-fly orchid) monitored during a long-term study of flowering and seed production (Whigham and O'Neill, 1988, 1991). Only four seedlings were observed over more than a decade and none of them lived for >2 yr (D. F. Whigham, unpublished data).

Great spatial and temporal variability in seedling establishment appears to be the rule in terrestrial orchids that have been studied to date. Tamm (1972, 1991) ob-

served great fluctuations from year to year and <5% recruitment into populations of the four orchid species that he observed for >50 yr in Sweden. Disappearances (presumed mortality) outnumbered recruitment so that population sizes decreased over time as Tamm's study sites were converted from meadows to forested habitats due to changes in land use practices. Vanhecke (1991) and Willem and Bik (1991) also found that recruitment was variable in space and time but that it was an important component of population maintenance of *Dactylorhiza praetermissa* (Druce) Soó and *Orchis simia* Lam. in face of environmental changes.

Seeds of terrestrial orchids are so small that practically nothing is known about their fate in the soil. When spontaneous seedlings of terrestrial orchids have been observed, they have most often been found close to roots of adult plants (Fabre, 1856; Ames, 1922; Mrkvicka, 1990), next to inflorescences that had fallen over but were still attached to parent plants (Bernard, 1899), and in pots containing cultivated plants (Beau, 1920; Wooster, 1935). Establishment of the mycoheterotrophic seedlings near adult plants would be expected, given the proximity of both seed source and a potential seedling symbiont among the fungi associated with the roots of the adult plants.

In a few cases, reports of spontaneous orchid seedlings have been accompanied with descriptions of their associated substrate. Fuchs and Ziegenspeck (1926) and Curtis (1943) found seedlings of *Cypripedium calceolus* L. growing in partially decomposed plant matter below a layer of living moss. In *Platanthera blephariglottis* (Willd.) Lindl. and *Dactylorhiza fuchsii* (Druce) Soó seedlings have been found growing in association with mosses (Case, 1964; Leeson, Haynes, and Wells, 1991), and seedlings of *Orchis ustulata* L., *Epipogium aphyllum* Sw., and *Corallorhiza trifida* Chat. were observed in humus and decomposing litter (Irmisch, 1853; Stojanow, 1916; Fuchs and Ziegenspeck,

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1924). A high humus content in the germination substrate seems to be typical, not only in species such as *E. aphyllum* and *C. trifida* that are highly dependent on mycotrophy, but also in species that produce a substantial amount of green foliage.

Seedlings of *Tipularia discolor* have been observed on decomposing logs, stumps, and piles of wood chips (Rasmussen, 1992) that had been dumped along trails and roads through the same forest where we have been conducting a long-term population study (Whigham and O'Neill, 1988, 1991). Based on a limited number of observations and literature reports we hypothesized that germination and seedling establishment of some terrestrial orchids are associated with deposits of organic debris and, more specifically, that the germination of *T. discolor* depends on decomposing wood. The latter contention is the focus of this paper.

Tipularia discolor is widespread throughout the southeastern United States and is common in successional and mature forests at the Smithsonian Environmental Research Center (SERC). It is one of the few woodland species that produces leaves (usually one per plant) in the autumn which senesce in late spring (i.e., wintergreen). Plants are leafless during the summer. Flowering occurs in the last few days of July and the first 2 wk of August at SERC and fruits mature throughout the late summer and autumn. Seeds typically disperse in late October and early November following the first freezing temperatures.

MATERIALS AND METHODS

Study site—The research was conducted at the Smithsonian Environmental Research Center (SERC), which is located on the Inner Coastal Plain of Maryland (USA) at 38°53'N, 76°33'W. The study areas were in mature and successional deciduous forests typical of the Inner Coastal Plain. Dominant tree species are *Fagus grandifolia* Ehrh., *Quercus alba* L., *Quercus falcata* Michx., *Liriodendron tulipifera* L., *Liquidambar styraciflua* L., *Carya glabra* (Mill.)Sweet, *Carya tomentosa* (Poir.)Nutt. *Cornus florida* L. and *Carpinus caroliniana* Walt. are the dominant understory trees and the woodland herb flora is diverse in the mature forest (Whigham, O'Neill, and Cipollini, 1993).

Field observations of seedlings—During the autumn and winter of 1991–1992 we conducted extensive searches for seedlings of *T. discolor*, primarily on decomposing logs and branches, stumps, and piles of wood chips. When seedlings were located, we attempted to identify the species of the decomposing material, usually by means of bark identification. Each plant that was found was excavated to determine whether it was a seedling, i.e., with a persisting protocorm, or a small established plant. This distinction was important because individuals can revert to very small sizes in response to disturbances such as herbivory (Whigham, 1990). We also conducted a detailed search for seedlings in the soil that was adjacent to two decomposing logs where seedlings were found. Two 1 × 10 m plots were established, which were oriented at right angles to each log.

Sowing experiments in situ—Seeds for the 1991 experiment were collected during the last week of November at SERC and kept at room temperature for 2–3 wk following harvest. Seeds were placed into seed packets (30–280, on average 120 per packet, as in Rasmussen and Whigham [1993]), which were placed horizontally in the surface soil at a depth of <10 mm. Eleven packets were sown at each of three plots in each of three forested sites where adult plants occurred. From each plot one seed packet was retrieved every 6 wk until July 1992 and after

that at varying intervals until the experiment was terminated in August 1993. Seed packets were examined for germination with a stereomicroscope either immediately or after storage in 70% ethanol. Germination was defined as rupture of testa associated with a swelling of the embryo.

Seeds used in the 1992 experiments were collected on 5 November and placed into seed packets, which were stored in plastic bags in the refrigerator (~5°C) until they were sown in two sites on 10 November. One site was a successional forest, ~50 yr old, dominated by *Liriodendron tulipifera* and *Liquidambar styraciflua*, the other was a mature forest, >125 yr old, which had been the site of our previous research on *T. discolor* (Whigham and McWethy, 1980; Whigham, 1984, 1990; Whigham and O'Neill, 1988, 1991; Zimmerman and Whigham, 1992). Wood was removed from a highly decomposed, unidentified stump on which abundant *T. discolor* seedlings had been found. This wood was homogenized lightly by hand and all objects above ~10 mm in length were removed. At each site we established nine plots (0.5 × 0.5 m) by removing the leaf litter from an area and then excavating the soil to a depth of 10 cm. These plots were randomly assigned to three treatments: three plots were refilled with excavated soil, three were filled with a 1:1 mix of excavated soil and decomposing wood, and three were filled with decomposing wood. Seed packets were placed horizontally in each plot at a depth of 5 cm. Beginning 24 wk after sowing, one seed packet was retrieved from each plot at intervals of 12 wk for the first three harvests, then at irregular intervals until December, 2 yr after sowing. Germination percentage of harvested seed packets was recorded with a stereomicroscope and lengths of protocorms were measured with an eyepiece gradicule at a magnification of 50×. When many seedlings were present, we measured the lengths of ten randomly sampled individuals. Treatment effect on the number of seed packets with germinated seeds were compared with chi-square tests.

RESULTS AND DISCUSSION

Spontaneous seedlings—The youngest observed protocorms of *T. discolor*, in early autumn, were pea shaped with a small shoot tip (Fig. 1), later to become typically bean shaped, ~7 × 10 mm. The first leafy shoot arises from the meristem on the concave side of the “bean” and consists of one-to-several scale leaves and a single, less than 5 mm broad foliage leaf. This leafy shoot appears later in the autumn and persists throughout the winter and senesces in spring. Larger protocorms (perhaps passing several seasons underground) occur and can be variously lobed with several growth points (Rasmussen, 1995; Fig. 7.1). Larger protocorms are able to send up more than one leafy shoot, thus initiating multiple sympodia that could ultimately lead to vegetative reproduction (Fig. 2). From below one of the lowermost nodes the first root is produced and during winter the nodes swell, eventually giving rise to the first corm, ~10 × 10 mm (Fig. 3).

We observed *T. discolor* seedlings growing on decomposing wood at 24 sites in the forest (~20–30% of the places that were searched), mainly on logs lying on the ground or stumps (Figs. 4–5). The decomposing wood that could be identified belonged to seven different deciduous tree genera/species and one conifer (Table 1). Seedlings were most common on *Liriodendron tulipifera*. The data suggest that suitable habitats for *T. discolor* seedlings are provided by a range of species that are common in mature and successional habitats. No seedlings were found on soil in the two 10 × 1 m plots that were searched near logs, nor were any seedlings noted elsewhere on soil during the observation period.



Figs. 1-5. Seedlings of *Tipularia discolor* and their habitat. 1. Two seedlings before shoot development (September). Arrow: shoot tip. Scale bar = 2 mm. 2. Lobed protocorm (P-P) with two shoots developing (S). At base of shoots, first root (R) coming through lowermost scale leaf. Scale bar = 4 mm. 3. Unusual seedling in spring (April). Above the protocorm (P) several roots and a corm (C) have developed at the base of shoot. Scale bar = 13 cm. 4. Stump of oak (*Quercus alba*) in mature forest with seedlings in situ. Arrow: site of Fig. 5. 5. Seedlings in winter (January) on top of stump, the protocorm buried in a mixture of moss and decomposing wood.

TABLE 1. Habitats for seedlings of *Tipularia discolor*. Each record represents one identifiable decomposing log or stump on which protocorms were found.

Species	Number of records
<i>Acer rubrum</i>	1
<i>Carya</i> sp. (<i>glabra</i> ?)	1
<i>Liquidambar styraciflua</i>	2
<i>Liriodendron tulipifera</i>	5
<i>Pinus virginiana</i>	1
<i>Prunus serotina</i>	2
<i>Quercus alba</i>	4
<i>Quercus</i> sp.	1

Germination in seed packets—In the 1991 experiment no germination was recorded during the 20 mo of observation. In the 1992 experiment, seeds in some of the seed packets germinated 7 mo after sowing (Fig. 6). Seeds germinated in two seed packets in unamended soil at the mature forest site (out of 36); all the rest of the germinated seeds were found in packets that had been placed in decomposing wood (11 of 36) or the 1:1 wood-soil mixture (six of 36). In both successional and mature forest, packets with germinated seeds were more frequent in amended substrate than in ambient soil, and in the successional forest the difference was statistically significant ($P < 0.015$). This result indicates that some factor in the decomposing wood stimulated germination; the two successful seed packets in ambient soil could have occurred simply because of a natural occurrence of some decomposing wood within the mature forest plots.

Seed quality and handling before sowing could be an explanation for the difference in germination between the two experiments (Rasmussen, 1995). The complete failure of germination in the 1991 experiment could be due to poor seed quality in that year, or to low seed viability after storage at room temperatures for 2–3 wk; in the 1992 experiment the seeds were stored in a refrigerator and sown almost immediately. However, the fact that seeds germinated in only two packets in unamended soil in the 1992 experiment, while at the same time germination occurred widely in the amended plots shows that the presence of the decomposing wood was decisive for germination. It is thus possible that seeds used in the 1991 experiment were equally viable but unable to germinate in the ambient soil.

We did not find any trend of increasing germination percentage over time, nor did the length of the seedlings increase (data not shown). This suggests that the protocorms, although often infected, had not obtained a functional mycorrhiza. The protocorm growth would then be limited to the amount of resources available within the seed reserves. Although the content of the decomposing wood or the conditions that it created stimulated germination, either organisms or conditions were unfavorable for the establishment of mycorrhiza. Since spontaneous seedlings of *T. discolor* were growing in the decomposing wood shortly before it was used in the experiment, it should have contained symbionts required for seedling growth. However, our processing of the wood for soil amendment may have inadvertently killed the symbiont. Another possibility is that the physical/chemical condi-

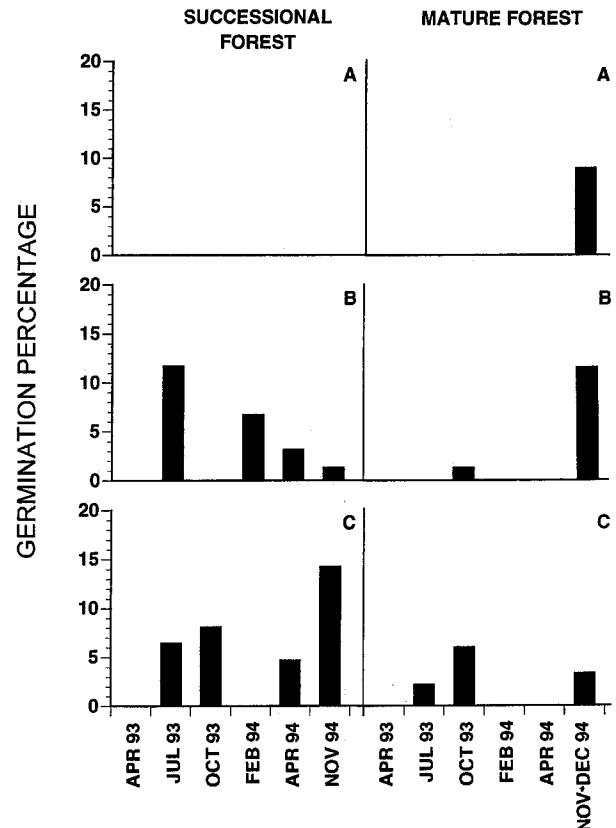


Fig. 6. Germination of *Tipularia discolor* in seed packets planted in successional (left column) and mature forest (right column). Each bar represents an average of three packets. (A) Unamended, ambient soil. (B) Mixture of ambient soil and decomposing wood (1:1 w/w). (C) Decomposing wood.

tions created in the sowing plots were unsuitable for establishment of mycorrhiza.

An interesting observation of the second experiment was the large amount of spatial and temporal variation in seed germination. In no instance did we find seed germination in all three seed packets that were collected from each site each time (Fig. 6); in instances where seeds had germinated in two seed packets, differences in germination percentage were usually large (data not shown). This indicates a large amount of spatial variability in the environmental factors that determine germination, which is difficult to explain since the substrate was thoroughly mixed before sowing.

This is one of the first in situ studies of seed germination in terrestrial orchids (Zettler, 1996) and the first to provide clear evidence that seed germination of a terrestrial orchid can be enhanced by the presence of decomposing wood. Based on our findings and the few literature reports cited above, decomposing wood and other organic matter should be investigated more widely as a requirement for germination of terrestrial orchid species, other than *T. discolor*. Organic debris could be an important factor in designing successful restoration efforts, for instance of *Cypripedium* spp. The results also emphasize the ecological importance of coarse woody debris, the

source of most decomposing biomass in forest ecosystems, going beyond the maintenance of carbon and nutrient standing stocks (Harmon et al., 1986). Decomposing wood is an important habitat for establishment of tree seedlings in forests in the Pacific Northwest and for fungal sporocarps that grow on wood and provide food for a wide range of animals (North, Trappe, and Franklin, 1997). This study provides evidence that the ecology of understory plants may also be directly linked to decomposing wood.

Tipularia discolor is furthermore interesting as an example of a woodland species that depends on the turnover of wood for population maintenance. Each individual experiences a change in habitat during establishment; as the plants mature, the woody debris on which they germinated is decomposing and the adult plants eventually grow in soil with a much lower organic matter content. The change not only of habitat but also of nutrition, i.e., a transfer from a mainly mycoheterotrophic seedling to a supposedly mainly photosynthetic flowering stage, could be associated with a change in symbiont preferences. Fungal switching during the lifetime of an orchid has never been documented, but the observations of Zelmer, Cuthbertson, and Currah (1996) suggest that some symbionts play an important role in orchid seedling establishment while being largely replaced by others in the adults. In *T. discolor* such a switch seems very possible considering the change of substrate during the life history. In view of the habitat of the seedlings, which we have described here, the fungi, involved most likely are saprophytes. Fungi associated with the flowering individuals could be either saprophytes, ectomycorrhizal fungi, or parasites on other plants. We have isolated at least two different kinds of symbionts from the roots of young plants (unpublished data); those from protocorms invariably were *Rhizoctonia*-like fungi with moniloid cells, although slow-growing in asymbiotic culture. Similar strains were obtained from roots, but on two occasions, isolates from the roots of a young seedlings were dark, nonrhizoctoneous mycelia. Recent DNA analysis (L. Taylor, unpublished data) of isolates from roots of mature *T. discolor* collected at SERC suggest that they are ectomycorrhizal.

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