termination, and parallel ethylene controls were always determined.

During the first 9 days in culture the modified fungus did not reduce acetylene. Acetylene reduction was detected 17 days after isolation and the activity peaked on day 29 to 30 (Fig. 1A). After that time there was a gradual decrease in activity, until on day 55 there was no activity. Upon transference to fresh nitrogen-deficient Hagem's medium 61 days after isolation there was further activity, which lasted 6 to 7 days and peaked on day 3. This reappearance of acetylene reduction was repeated after 93 days in culture. After 138 days, protoplasts were again produced by enzymic digestion of the cell walls. The protoplasts were plated on nitrogen-deficient Hagem's medium and acetylene reduction was detected 72 hours later. The unmodified *Rhizopogon* mycelium at no time either reduced acetylene or produced detectable ethylene during the course of these experiments, and the modified form did not produce ethylene in the absence of acetylene.

*Azotobacter* on nitrogen-deficient Hagem's medium had a peak of acetylene reduction activity 5 days after plating, whereas no activity was detected in the fungus until 17 days after isolation.

The optimum *p*H for acetylene reduction by *Azotobacter* was 8, whereas in the fungus optimum reduction occurred at *p*H 4, near the optimum *p*H for the growth of *Rhizopogon* (3.8). Assays of $^{15}N$ have confirmed fixation of nitrogen with an acetylene/nitrogen ratio of 5.3 (*6*).

The modified fungal strains, unlike the control fungus, were capable of growing on media free from available combined nitrogen, including media based on silica gel. The modified strains were capable of growth and acetylene reduction on nitrogen-free media containing either streptomycin sulfate (50 $\mu$g/ml), tetracycline (50 $\mu$g/ml), or benzyl penicillin (400 $\mu$g/ml). *Azotobacter* neither reduced acetylene nor grew on these media. It is unlikely that lysozyme and penicillin treatments would enable *Azotobacter* to survive outside the fungal mycelium and reduce acetylene in the presence of these antibiotics, especially in the absence of an osmoticum.

Electron microscopy of the hyphae of the modified strains showed in each case the inclusion of spherical bodies 0.5 $\mu$m in diameter (Fig. 1C). Such bodies were absent from the wild-type fungus. The bodies enclosed a series of lamellar structures and vesicles characteristic of vegetative cells of *Azotobacter* but lacked any trace of a cell wall. We consider that these bodies represent either modified mitochondria, possibly indicating the location of the bacterial genetic information after transgenosis (*7*), or L-forms of the bacteria, the loss of the cell wall being due to either digestion by the fungus or the action of the various antibiotics used to decontaminate the cultures. The L-forms are enclosed by an extra membrane (Fig. 1D), whereas in a study of chloroplast uptake by fungal protoplasts (*8*) no extra membrane was found.

No cyst formation was observed in the fungal hyphae but the finding of frequent pairs of the modified mitochondria or L-forms in thin section suggests that division does occur. Poly-$\beta$-hydroxybutyric acid, the storage product of *Azotobacter* cysts, was found in the modified though not in the wild-type fungal hyphae. It was membrane-bound but occasionally merely included in the fungal cytoplasm.

These results strongly indicate the possibility of directed transgenosis for nitrogen-fixing ability to eukaryotic cells, although the mechanism behind its operation is obscure. An interesting parallel to these findings exists in the report of a forced symbiotic association between cultured cells of carrot and cells of *A. vinelandii* (*9*). The location of the bacterial genetic information and whether the modified fungus is still capable of a mycorrhizal relationship with *P. radiata* remain to be determined.

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### References and Notes

1. Hagem's medium consists of 9.4 m*M* $NH_4Cl$, 3.4 m*M* $KH_2PO_4$, 2 m*M* $MgSO_4 \cdot 7H_2O$, 1 ml of 1 percent $FeCl_3$, 5 g of glucose, 5 g of malt extract, and 1.5 percent agar per liter; the *p*H is 4.7.
2. O. M. H. de Vries and J. G. H. Wessels, *Antonie van Leeuwenhoek J. Microbiol. Serol.* **39**, 397 (1973).
3. Ashby's medium consists of 3.2 m*M* $K_2HPO_4$, 2 m*M* $MgSO_4 \cdot 7H_4O$, 0.1 m*M* $FeSO_4 \cdot 7H_4O$, 0.1 m*M* $MnSO_4 \cdot 2H_2O$, 0.04 m*M* $Na_2MoO_4$, 1.7 m*M* NaCl, 5 m*M* $CaCO_3$, 5 g of glucose, and 1.2 percent agar per liter; the *p*H is 8.0.
4. H. T. Bonnett and T. Erikssen, *Planta* **120**, 71 (1974).
5. Here KCl replaced $NH_4Cl$, and the malt extract was replaced by nicotinic acid (0.5 mg/liter), pyridoxine-HCl (0.1 mg/liter), and thiamine-HCl (0.1 mg/liter).
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# Role of *Erythronium americanum* Ker. in Energy Flow and Nutrient Dynamics of a Northern Hardwood Forest Ecosystem

Abstract. *The aboveground activity of the spring herb,* Erythronium americanum, *is restricted to the period between snowmelt and forest canopy development. Its phenology and production capacity closely adapt the species to this temporal niche in northern deciduous forests. While* E. americanum *has a minor effect on energy flow, it may reduce losses of potassium and nitrogen from the ecosystem during the period of maximum removal by incorporating these elements in accumulating biomass. Later, during the summer, these nutrients are made available when the aboveground, nonperennating tissues decay.*

Intensive biogeochemical studies of the Hubbard Brook Experimental Forest in central New Hampshire (*1*) provide a framework for evaluating the contribution of individual species to energy flow and nutrient dynamics in the northern hardwood forest ecosystem. Deciduous trees constitute > 97 percent of the living biomass and exert a primary influence on ecosystem functions (*2*). However, deciduous trees are summer-green species and their most active period is from late May to mid-October. Vernal photosynthetic herbs, primarily *Erythronium americanum* Ker. (Liliaceae), are abundant in the ecosystem and are active during the period between snowmelt and development of the overstory forest canopy. This is a time of high light intensity at the forest floor, warm and moist soil conditions, and increased nutrient availability. During this period, *Erythronium* is the dominant autotroph. This study was initiated to evaluate its role in ecosystem energetics and biogeochemical cycling (*3*).

In 1972 and 1973, phenology and biomass accumulation of the *Erythronium* population were measured on a 13.2-ha watershed. This watershed (W6) serves as a reference ecosystem for the Hubbard Brook study and its biogeochemistry and ecology are extensively known (*1*, *4*, *5*). For biomass estimates, the watershed was divided into five elevational strata. Average weights per plant and per plant part were obtained from harvests within each stratum and were combined with density data to estimate biomass of the population on the watershed at given harvest times. Separate harvests of both above- and belowground parts were made for determination of calcium, magnesium, potassium, and nitrogen concentrations and these values were combined with biomass data to obtain estimates of the total content of these elements in the population (*6*).

Spring growth of *Erythronium* exhibited an initial lag phase during which little or no net biomass accumulation occurred (Fig. 1). This phase lasted for about 15 days in both years and corresponds rather closely with the early period of shoot development following snowmelt. After this lag phase, biomass accumulation occurred rapidly. Average plant weight increased from 42 to 119 mg during 16 days in 1972, and from 33 to 149 mg during 42 days in 1973. However, most (85 to 90 percent) of this accumulation occurred within 11 days (25 percent of the period of aboveground activity) in 1972 and 21 days (40 percent) in 1973. Net biomass increases were 1.8 and 3.5 times the initial spring biomass in the 2 years, respectively. Estimates of net photosynthetic rates calculated from the biomass accumulation curves were 18.5 mg of $CO_2$ per gram (dry weight) per hour in 1972 and 26.0 mg $g^{-1}$ $hour^{-1}$ in 1973. These rates correspond with values determined by gas exchange techniques and are high in comparison with those for other herbaceous species of the deciduous forest, particularly summer-green species (*7*). Net spring production of the population on the watershed was 11.3 g $m^{-2}$ $year^{-1}$ in 1972 and 16.3 g $m^{-2}$ $year^{-1}$ in 1973.

Table 1. Net uptake during spring growth and net release of nutrients due to shoot decomposition of *E. americanum* compared to gross spring stream water losses (April and May) from the ecosystem and gross annual losses.

| Year | Net uptake (kg/ha) | Net release (kg/ha) | Stream water losses (kg/ha) | |
|---|---|---|---|---|
| | | | Spring | Annual |
| | | *Calcium* | | |
| 1972 | | 0.1 | 6.1 | 10.7 |
| 1973 | 0.1 | 0.2 | 3.6 | 14.0 |
| | | *Magnesium* | | |
| 1972 | | 0.1 | 1.3 | 2.6 |
| 1973 | 0.1 | 0.1 | 0.8 | 3.2 |
| | | *Potassium* | | |
| 1972 | | 0.9 | 1.1 | 1.7 |
| 1973 | 0.6 | * | 0.7 | 2.4 |
| | | *Nitrogen* | | |
| 1972 | | 1.9 | 2.9 | 3.8 |
| 1973 | 1.0 | 0.9 | 1.1 | 4.3 |

*In 1973, there was a net gain in total potassium in the biomass of *Erythronium* during senescence.

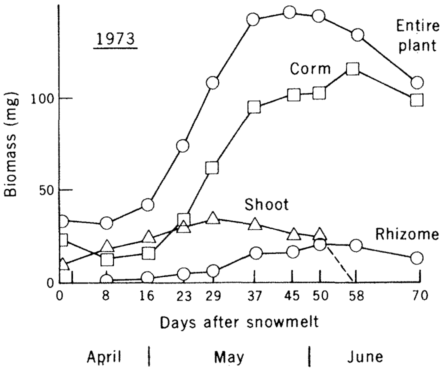

Fig. 1. Average biomass per plant and plant part of *Erythronium americanum* in 1973. The 1972 data exhibited similar growth characteristics.

Based on the data from combined production studies at Hubbard Brook, the aboveground net production of all vascular plants has been estimated as 957 g $m^{-2}$ $year^{-1}$ (*3, 5, 8*). Most of this (94 percent) was accounted for by summer-green tree species (primarily sugar maple, beech, and yellow birch). Including herbs, summer-green species accounted for 96 percent of the aboveground production, evergreen species for 3.5 percent, and vernal photosynthetics for 0.5 percent. This distribution of production parallels that of beech forests at high elevation (1580 m) in the Great Smoky Mountains of North Carolina and Tennessee which occupy approximately the same elevational position in the transition from deciduous to boreal forests as the Hubbard Brook forest (*9*).

In the undisturbed deciduous forest ecosystem strong biotic mechanisms exist which reduce loss of nutrients in stream water (*10*). However, most of this influence is restricted to the summer months. Greatest losses of nutrients occur during the spring months, when snowmelt runoff is high and the influence of summer-green species (transpiration and nutrient uptake) is low (*1, 10*). During this period nitrate concentrations in soil and stream water are elevated over summer values (*11*).

The rapid spring growth of *Erythronium* occurs when light intensity at the forest floor and soil nitrogen availability are high and coincides with the final stages of high spring runoff and nutrient loss (Fig. 2). Senescence of *Erythronium* shoots occurs after canopy development, when stream discharge is low and nutrient losses from the ecosystem are correspondingly reduced. This temporal pattern suggests that *Erythronium* may act as a short-term sink, or "vernal dam," with nutrients incorporated in the accumulating biomass during the spring flushing period and released by shoot decomposition during the early summer. Such a mechanism would reduce losses in spring stream flow and preserve the nutrient capital of the ecosystem.

To test this hypothesis, we calculated release of calcium, magnesium, potassium, and nitrogen from the above- and belowground biomass of *Erythronium* during early summer in 1972 and 1973 and uptake of these elements during the spring growth period in 1973. The estimates of uptake and release are compared with nutrient export from the ecosystem in stream water during April and May (the period of *Erythronium* activity) and during the entire water year, June through May (Table 1) (*12*).

The uptake and release of calcium and magnesium are small in comparison to stream water losses in April and May, indicating that *E. americanum* has little effect on the biogeochemistry of these elements. In contrast, the potassium and nitrogen cycles both appear to be strongly affected by *E. americanum*. In 1973, the amounts of potassium and nitrogen incorporated in the increasing *Erythronium* biomass were approximately 86 and 91 percent of the amounts lost in stream water during April and May, and 32 and 24 percent of annual stream water losses, respectively. Releases of potassium and nitrogen due to shoot decomposition in 1972 were 82 and 67 percent of spring stream water losses and 53 and 50 percent of annual losses. Additional release of these elements from *Erythronium* biomass may be assumed to occur through-

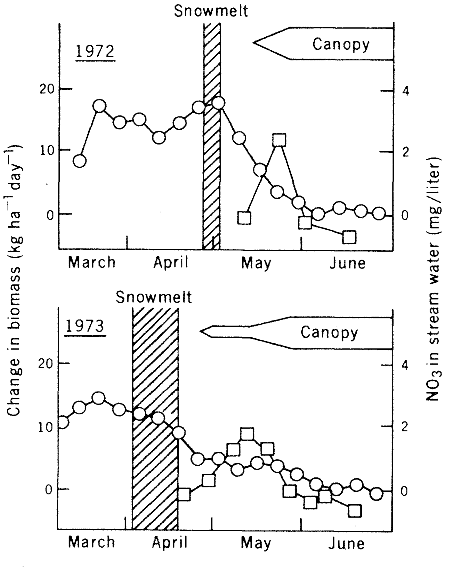

Fig. 2. Stream water nitrate concentrations (○) and biomass increase in *Erythronium americanum* (□) in 1972 and 1973. Biomass increase was calculated as the rate of biomass change between any two consecutive harvest dates.

out the remainder of the summer period due to decomposition of rhizomes and death of underground perennating organs (*3*).

The data presented here indicate that significant amounts of potassium and nitrogen were retained in accumulating *Erythronium* biomass in early spring and made available through shoot decomposition in midsummer. The vernal dam hypothesis implies that had *E. americanum* not been present, nutrients in amounts equivalent to those taken up in its biomass and subsequently released by decomposition would have been subject to spring stream water flushing. This interpretation does not necessarily imply ecosystem evolution; however, it does illustrate how the adaptation of one species to a period of high nutrient availability may benefit the ecosystem through reduction of nutrient losses. Thus, even though *E. americanum* accounts for a very small proportion of the net primary production of the ecosystem, it may significantly influence the nutrient dynamics of the system.

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4. The Hubbard Brook forest is an approximately 60-year-old stand composed primarily of sugar maple (*Acer saccharum*), beech (*Fagus grandifolia*), and yellow birch (*Betula alleghaniensis*). For a complete description of the study area see Muller (*3*); Siccama *et al.* → F. H. Bormann, T. G. Siccama, G. E. Likens, R. H. Whittaker, *Ecol. Monogr.* **40**, 373 (1970).

→ T. G. Siccama, F. H. Bormann, G. E. Likens, *Ecol. Monogr.* **40**, 389 (1970).

6. Weekly biomass harvest consisted of five samples of 25 entire plants each in each of the five elevational strata. Harvests for chemical analyses were made approximately every 2 weeks and consisted of nine samples of about 100 plants each.
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11. Soil nitrate concentrations remain at high winter levels through April and approach low summer levels in May (J. Mellilo, personal communication). These values are reflected in stream water nitrate concentrations.
12. While the data were not available to calculate uptake in 1972, the similarity of uptake and release of calcium, magnesium, and nitrogen in 1973 suggests that the release values in 1972 are adequate indicators of uptake. Data for spring and annual stream water losses were provided by J. Eaton and G. E. Likens (personal communication).
13. This is a contribution of the Hubbard Brook Ecosystem Study, funded by the National Science Foundation. Preparation of the manuscript was supported by the Energy Research and Development Administration. We thank B. Green, T. Green, P. Marks, J. Mellilo, R. Pierce, T. Siccama, D. Sprugel, and G. Whitney for helpful comments on the manuscript.

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## Dinoflagellates: Fossil Motile-Stage Tests from the Upper Cretaceous of the Northern New Jersey Coastal Plain

Abstract. *Fossil dinoflagellate tests have been considered to represent encysted, nonmotile stages. The discovery of flagellar porelike structures and probable trichocyst pores in the Upper Cretaceous genus* Dinogymnium *suggests that motile stage tests are also preserved as acid-resistant, organic-walled microfossils.*

The life cycle of living dinoflagellates has been shown, in some cases, to consist of a motile planktonic stage and an encysted, nonmotile benthonic stage (*1*). Dinoflagellate fossils have been thought to represent only the encysted, nonmotile stage, with the fossil itself being the cyst. To my knowledge, this report is the first discussion of morphological features (flagellar pores and probable trichocyst pores) which suggest that motile stages are also preserved as fossil dinoflagellates.

The only genus of fossil dinoflagellates that has been considered to be possibly of the motile stage is *Dinogymnium* Evitt *et al.* 1967. It was noted by Evitt (*2*) and Evitt, Clarke, and Verdier (*3*) that the cystlike tests of this genus bore morphological features that resembled those of motile stages. These features include wall canals (interpreted here as trichocyst pores) that penetrate the test wall, and a cingulum and sulcus that never appear to be interrupted by transverse structures that would inhibit the operation of a flagellum along their length. Also, fossil *Dinogymnium* tests show a striking similarity in morphology to the tests of living motile stages of the genera *Gymnodinium* Stein and *Gyrodinium* Kofoid and Swezy, which suggests that *Dinogymnium* tests may represent ancestral motile stages. Flagellar pores, however, are one of the features that must be present on a fossil form before it can be proven to be a motile stage. Until now, no such pores have been reported for *Dinogymnium* or for any other dinoflagellate.

Flagellar pores on modern dinoflagellates occur at the intersection of the cingulum, a transverse furrow, and the sulcus, a longitudinal furrow, and mark the exit of the transverse flagellum (which is restricted to the cingulum) and the longitudinal flagellum (which is restricted to the sulcus). Scanning electron microscopy of fossil *Dinogymnium* specimens

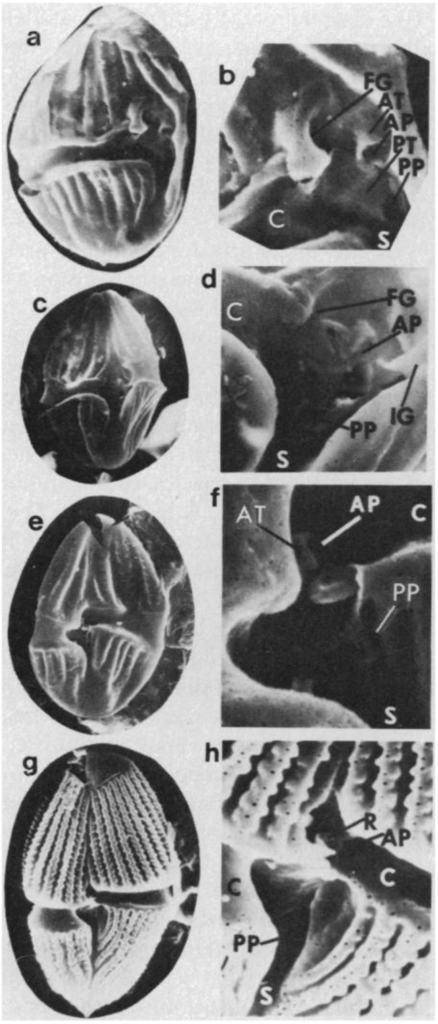

Fig. 1. The left column (a, c, e, and g) shows complete ventral views of *Dinogymnium* tests. Enlargements to the right (b, d, f, and h) show cingulum-sulcus intersections with flagellar pore structures; anterior flagellar tube (*AT*) and pore (*AP*) allowed passage of the transverse flagellum onto the cingulum (*C*); posterior tube (*PT*) and pore (*PP*) allowed passage of the longitudinal flagellum onto the sulcus (*S*); *IG*, initial grove; *FG*, final groove; *R*, half-circular ridge.