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Mammalian epidermal growth factor promotes plant growth

(herbivores/sorghum seedling bioassay)

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ABSTRACT **Application of mouse submaxillary gland epidermal growth factor to young sorghum seedlings at low concentrations (≈0.4–4 μM) increased shoot growth significantly over 3- and 6-day periods. The effects were dose dependent.**

Studies of the effects of herbivores on plant function most often have emphasized predation rather than commensalism (1). Recently, however, this attitude has changed with accumulating evidence that herbivores may stimulate plant growth processes (2–6) and may enhance plant fitness (7, 8). Mattson and Addy (2), Owen and Wiegert (7), and Stenseth (8) view the association as indirect when processes such as nutrient cycling are altered following herbivory. Others suggest more direct effects. McNaughton (6) summarized nine points which include both indirect and direct effects; among the latter he included effects on plants which arise as a function of biochemicals being deposited during the herbivory process, although he questions whether grazing does anything but decrease fitness. Earlier, others considered saliva and its various compounds which might affect plant growth (9–13), but there is no general acceptance of the fact that saliva left in wounded plant tissue might dramatically affect plant growth. Nonetheless, for many species herbivory promotes plant growth at first, followed by general decline in productivity once the level of herbivore attack passes the point where the plant can no longer compensate for lost tissue. This phenomenon is termed the "herbivore optimization concept" and follows the descriptions of Dyer (3) and McNaughton (6).

Even though mechanical pruning may alter plant growth and development, mostly from changes in photosynthesis (14, 15), there is yet the nagging question of whether that is the whole story. Experiments in which herbivory has been simulated through clipping with additions of whole saliva are ambivalent (10, 16) or show mixed photosynthetic response (17). To cut through difficulties dealing with this issue, experiments were conducted to test the hypothesis that growth factors in vertebrate saliva (18–20) affect plant growth. Mouse submaxillary gland epidermal growth factor (mEGF) was chosen for the initial tests.

METHODS

Lyophilized mEGF (purity >95% as determined by NaDodSO$_4$/polyacrylamide gel electrophoresis; lot 867-66) was obtained from Collaborative Research (Waltham, MA) and handled according to recommendations. Experiments were conducted 29 May, 12 June, and 13 July in 1979. For the two treatments in Exp. 1, 3 and 30 μg of mEGF in 3 ml of distilled H$_2$O were added to Rio variety† grain sorghum (*Sorghum bicolor*) seedlings that had been germinated 3 days earlier and held in petri plates with sterile cotton substrate and 10 ml of full-strength Hoagland's solution (21) containing minute quantities of iron chelate; nine seedlings were used per petri plate. For Exps. 2 and 3, only the 30-μg treatment was used. Equal numbers of untreated controls were used throughout. At days 3 and 6, total shoot length of each seedling was measured to the nearest millimeter. All plants were held at 26°C under constant fluorescent lighting. A total of 117 seedlings was used during the three experiments. A two-way analysis of variance

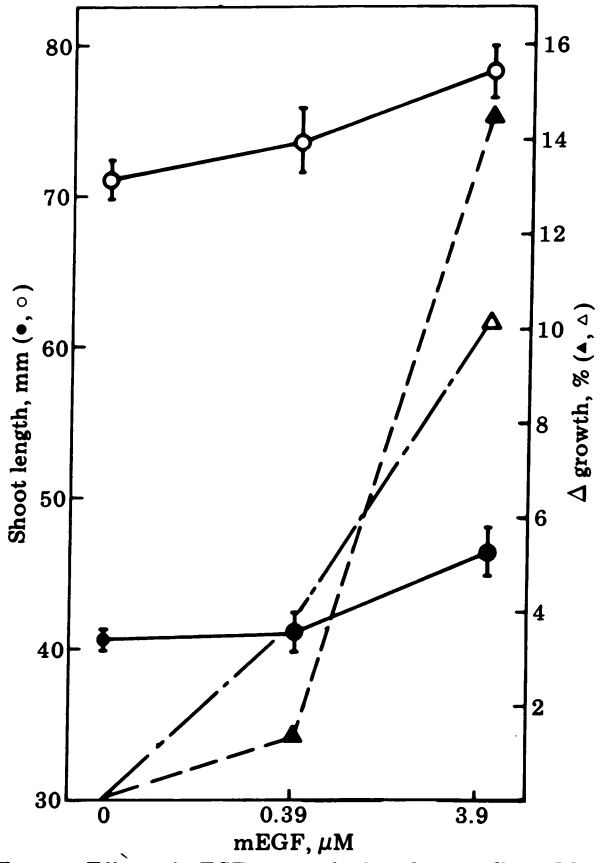

FIG. 1. Effects of mEGF on growth of sorghum seedlings. Means ± SEM are shown for days 3 (●, ▲) and 6 (○, △); nine seedlings per test were used. Treatment differences were significant ($P \leq 0.001$) according to two-way analysis of variance.

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Abbreviation: mEGF, mouse epidermal growth factor.

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† This is a sorghum variety grown for bioassay purposes at the U.S. Department of Agriculture, Beltsville, MD.

Table 1. Effects of treating sorghum seedlings with mEGF

	Shoot length, mm		
	Controls	With mEGF (3.9 μM)	% increase
Day 3	42.3 ± 0.50	46.0 ± 0.58	8.75
Day 6	75.5 ± 0.99	78.9 ± 0.94	4.50

Seedlings were treated 3 days after germination and then measured 3 and 6 days later to determine growth. mEGF-treated plants grew significantly in length compared to controls ($P \leq 0.05$); n for each group = 54. Results are mean ± SEM.

was used to test for dose effects; a randomized block analysis of variance, in which the blocks were replicated experiments, that accommodated uneven class and sample sizes was used to compare 30-μg treatments to controls.

RESULTS AND DISCUSSION

Exp. 1 indicated that mEGF increased growth in sorghum seedlings and that the reaction was dose related ($P \leq 0.001$) (Fig. 1). At 0.39 μM, mEGF increased growth 1.4% at 3 days and 3.8% at 6 days; mEGF at 3.9 μM resulted in 14.5% and 10.2% increases at days 3 and 6, respectively. Casual observation showed that at day 3 root growth, especially at 3.9 μM, was much more developed than for controls or the 0.39 μM group; this difference was less dramatic at day 6. Effects on root growth in Exps. 2 and 3 were not obvious. The analysis of variance of mEGF effects at 3.9 μM in all experiments showed that seedlings grew significantly longer than controls ($P \leq 0.05$) (Table 1), but there was no significant difference ($P \geq 0.05$) in growth rates between days 3 and 6. Thus, any effects imparted by mEGF must occur soon after treatment, in this case within 3 days.

These experiments show: (*i*) mEGF can stimulate plant shoot growth and possibly growth of roots as well, (*ii*) there is a dose-related response, and (*iii*) the effect is time dependent (i.e., there is a diminution of response relatively soon after plants have been exposed to mEGF). I suggest that this work provides new information about interrelationships between herbivores and plant function. EGF is found in salivary glands of several mammals, mainly in the submaxillary gland and in secretions that can be deposited upon the plant during feeding. EGF is also found in large amounts in mammalian urine (22). Some evidence, as yet unconfirmed, suggests that EGF-like material is found in the anterior part of the digestive system of insects; the exact location is not known to this point (unpublished data). And perhaps most important, EGF and related peptides are strongly mitogenic and stimulate protein production in low concentrations (18, 22). Thus, it seems reasonable that herbivores of many types have the capacity to influence plant growth-related processes in ways not now understood. If so, I pose the hypothesis that EGF or EGF-like compounds provide a basis by which herbivores may regulate plant community productivity and possibly play an important role in co-evolutionary processes as well.

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