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Fog drip: a mechanism of toxin transport from *Eucalyptus globulus*[1]

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DEL MORAL, ROGER, and CORNELIUS H. MULLER (University of California, Santa Barbara). Fog drip: a mechanism of toxin transport from *Eucalyptus globulus*. Bull. Torrey Bot. Club 96: 467–475. 1969.—Fog drip is a mechanism which leaches metabolites of many kinds from foliar crowns. In southern California few herbs of any species are found beneath the canopy of *Eucalyptus globulus*. Their absence proved not to be attributable to competition for essential resources; but rather allelopathic chemicals were found to be of principal importance. Natural fog drip was collected from beneath *Eucalyptus globulus* and shown by bioassay with *Bromus rigidus* to be inhibitory without concentration. Paper chromatography of fog drip revealed the phytotoxins chlorogenic, *p*-coumarylquinic, and gentisic acid. Artificial fog drip concentrated three fold inhibited several species of grasses in bioassays performed in artificial media. Fog drip was also toxic in soil bioassays. Additional allelopathic mechanisms in *Eucalyptus* involving terpenes adsorbed to soil colloids and phenolic acids leached from the leaf litter do exist, but *Eucalyptus globulus* fog drip acting alone appears to be capable of producing severe inhibition of growth in some herb species. The importance of fog drip as an agent of metabolite transfer from leaves and deposition in the environment lies in the capacity of these compounds to influence the structure and diversity of a plant community.

The capacity of trees and shrubs to collect fog drip is a well-known fact. Several workers have investigated the significance of fog, but nearly always in terms of moisture relations (Stone, 1963). Oberlander (1956) states that during a five-week summer period *Lithocarpus densiflorus* produced fog drip equivalent to 59 inches of rain. Shreve (1927) suggested that in the Santa Lucia Mountains the distribution of *Sequoia sempervirens* did not extend beyond the fog belt and that it was the presence of fog that permitted growth of redwoods southward along the California coast. On the other hand, Byers (1953) states that many luxuriant stands of redwood occur in the absence of significant summer fog. Stone (1957) presented experimental evidence that artificial mist (equivalent to either fog or dew) markedly prolonged the survival under drought stress of seedlings of *Pinus ponderosa*, *Libocedrus decurrens*, and *Abies concolor*. He suggested that

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the presence of regular fog might extend the distribution of some of these species. In some arid regions dew or fog seem to be responsible for the presence of a well-developed epiphytic flora (Waisel, 1958), and in less arid regions fog drip may influence the composition of the associated flora. This brief review indicates that there is some debate concerning the significance of fog to the moisture regime. However, we are aware of no previous study that has attempted to investigate fog drip as a chemical phenomenon. It is possible that the primary ecological significance of fog drip may lie not in changes in the moisture regime but rather in chemical effects. These chemical influences may be either stimulatory or inhibitory to the growth of plants and may substantially alter the structure and diversity of plant communities.

Tukey (1966) has shown that most, if not all, types of plants are subject to chemical losses by leaching when exposed to artificial fog. Foliar leachates from a large variety of plants contain many metabolites and minerals, including all essential elements, amino acids, carbohydrates, and organic acids. Growth regulators and alkaloids are also commonly encountered in various kinds of leachates. Quantities of these chemicals leached from a single plant may be great enough to supply another plant with all essential nutrients necessary for successful growth, development, and reproduction (Tukey and Mecklenberg, 1964). Tamm (1951) showed that minerals washed from a number of species were critical to the nutrition of their moss epiphytes. Thus the mechanism of leaching by rain or fog drip may affect the diversity and impose a distinct structure upon the plant community by altering the distribution of inorganic and organic chemicals within the system. Dominant populations may influence the ecosystem not only by purely physical or competitive means, but also by the action of inhibitory organic materials leached from intact, living plant organs.

Eucalyptus globulus Labill.[3] occurs throughout much of cismontane California both as a planted and as a naturalized species. It is particularly abundant in the Santa Barbara area, where, beginning in the 1870's, Ellwood Cooper made extensive plantings. The species is well suited to the Santa Barbara climate, being somewhat frost hardy and to a remarkable degree drought tolerant, considering its habit and native climate. This tree is smaller in California than the normal size in its native habitat in Tasmania (Penfold and Willis, 1961). It commonly attains a height of 30 m in California. Juvenile leaves are glaucous or gray while adult leaves are only moderately glaucous, dark green, and possess oil glands rich in terpenes. Adult leaves are linear, 10 to 30 cm by 3 to 4 cm, acute, narrowly lanceolate, and falcate. The stature of *E. globulus* and the shape and structure of its leaves result in its being an efficient interceptor of drifting fog (see Means, 1927, and Oberlander, 1956).

[3] Nomenclature of California species follows that of Munz and Keck (1963).

The leaf chemistry of *E. globulus* has been carefully investigated. Guenther (1950) states that cineole, α-pinene, and camphene are the important terpenes. This was confirmed for Santa Barbara material by del Moral (unpublished) using gas chromatographic techniques. In addition there is an array of phenolic acids present in the leaves. These include ellagic, gallic, caffeic, gentistic, *p*-coumarylquinic, and chlorogenic acids (Hillis, 1966). We have shown that some of these compounds are toxic to a variety of seed plants (del Moral and Muller, in press). The terpenes are very slightly soluble in water and the phenolic acids are water soluble. Beneath most stands of *Eucalyptus* an herbaceous understory is lacking or vastly modified. Thus it is reasonable to ask whether fog drip from *E. globulus* contains significant concentrations of toxins and whether they are capable of influencing the understory vegetation.

The pattern of inhibition. Most mature, undisturbed stands of *E. globulus* are virtually devoid of herbaceous annual species. The litter within these stands is often quite thick but diminishes rapidly beyond the canopy cover. Annual herbs gradually begin to appear and increase in height and density with increasing distance from the stand. Thus, in a typical transect, continuous litter extended 2.6 m from the bole and this area supported only a few scattered, stunted herbs. Stunted grasses were found from 3.0 to 6.0 m and fully developed grassland began at this point. Significantly, the crown of the trees also extended to the 6-m mark. The densest part of the crown, as visually estimated by the amount of sky seen from beneath, extended to the 3-m mark. The coincidence of the extent of canopy with such a herbaceous vegetative pattern has been repeatedly observed under a variety of edaphic and microclimatic conditions (del Moral, unpublished).

Mature *E. globulus* trees are abundant on the campus of the University of California, Santa Barbara. Most of these are well trimmed and the litter is periodically removed by raking so that little accumulates. Under such trees there is a paucity of herbs which suggests that, while litter is an important source of toxins in some *Eucalyptus* species (del Moral and Muller, in press), it is not necessary to the development or maintenance of herb inhibition in the case of *E. globulus*.

A further observation supports this conclusion. During the course of road construction on campus, large heaps of fresh top soil were piled beneath two trees of *E. globulus* which previously had maintained well defined zones of inhibition. During the subsequent growing season, this fresh soil supported a lush annual vegetation which, however, did not produce large quantities of seeds. In the two following years the rounded heaps accumulated no litter but nevertheless failed to sustain any herb growth. A similar series of events was observed several years earlier by the second author. Since the litter had not accumulated, the conclusion that litter is not necessary for this pattern to develop is mandatory.

During our investigations of several aspects of herb-*Eucalyptus* interactions, we have demonstrated that there were no edaphic reasons for the exclusion of herbs from *Eucalyptus* stands, that light and nutrients were adequate for herb growth (nutrient levels were nearly always greater within the stand than within adjacent grassland), and that small animals did not inhabit or visit *Eucalyptus* stands frequently enough to influence the herb vegetation (del Moral and Muller, in press). Soil moisture was nearly always as favorable within the stand as in grassland. On several occasions, particularly after a fog, soil moisture was greater within the stand than in the adjoining grassland. For example, in February, 1965, after a period of drought followed by a heavy fog, soil moisture in one stand was 12.5%. On the north side of the stand, 6 m away in grassland it was just 8.4%. A particularly luxuriant tree on the campus characteristically produced copious amounts of fog drip. The first branches were 4 to 5 m from the ground so that full sunlight easily penetrated diagonally to the bole. During the course of campus landscaping, a new lawn immediately adjacent to and on the southwest side of this tree was seeded, fertilized, and irrigated. The lawn developed rapidly, except beneath the crown of the tree where no germination occurred. This illustrates that *Eucalyptus* inhibition of herbs cannot be overcome by abundant nutrients or water. We concluded from our various observations and experiments that competition, edaphic conditions, and grazing could be safely excluded from among the important mechanisms of herb inhibition beneath *Eucalyptus*. It is recognized that some of these factors, particularly soil moisture competition, could act to limit herb growth under certain conditions.

Physiological effects of natural fog drip. Fog is common along the Santa Barbara coastline throughout the year. Heavy ground fogs may occur in nearly any month, but February, May and a period from August through November are particularly prone to such conditions (Elford, 1965). Coastal locations commonly receive considerable fog during the night and early morning hours. This fog often results in a heavy rain of drip beneath exposed trees. Since fog drip from *Eucalyptus* leaves could contain significant quantities of phytotoxins, an investigation of this potential was undertaken. Flasks capped with funnels were placed beneath trees adjacent to the Biology Building, University of California, Santa Barbara, when fogs were imminent. The material thus collected was filtered to remove any debris and subjected to the following bioassay technique. Seeds of *Bromus rigidus* were soaked for 2 hr in distilled water and planted on 5×5 cm sponges, 3 mm thick, covered with filter paper, and placed, 10 per dish, in 500-ml storage dishes. The sponges were moistened either with distilled water in the case of the controls or with the fog drip being assayed for toxicity. The chambers were sealed with parafilm and incubated in the dark for 48 hr at 26°C. *Bromus rigidus* was used as the primary bioassay species because it commonly occurs near *E. globulus*, germinates rapidly and uniformly, and grows quickly.

Sufficient fog drip for assay purposes was collected six times over a period of months and each collection was promptly assayed. The results of these bioassays, shown in Fig. 1, consist of mean radicle growth of *B. rigidus* in a fog drip sample expressed as percent of a simultaneous distilled water control. The narrow bars above each mean express the Honestly Significant Difference (Steel and Torrie, 1960) at the 5% level for each bioassay.

Each of the six trials of this experiment resulted in a significant reduction of growth. The large variation between trials is a function of the

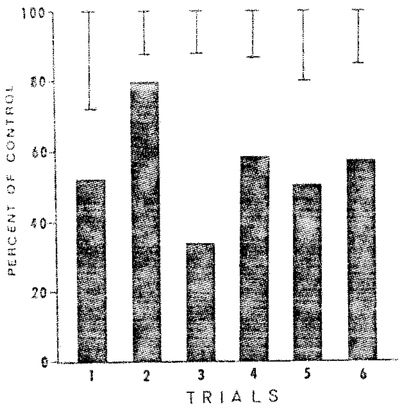

Fig. 1. Radicle growth of *Bromus rigidus* treated with natural fog drip collected from beneath *Eucalyptus globulus*. In each trial growth was compared to a simultaneous distilled water control. The thin bars represent the Honestly Significant Difference at the 5% level for each trial.

concentration of the fog drip collected on any particular occasion. When a great quantity fell, that collected by funnels was more dilute than if only sparse drip occurred. No attempt was made in this experiment to concentrate the solutions. Thus the experiment demonstrated conclusively that fog drip in its natural state indeed contains significant concentrations of toxic material.

Because of the uncertain supply of natural fog drip, additional bioassay experiments were performed utilizing artificially obtained material. Freshly collected leafy branches were suspended above a large plastic sheet attached to a wooden frame to form a funnel. A fine mist of distilled water was applied to the leaves at intervals until 1500 ml had been collected. This was concentrated to 500 ml under vacuum at 45°C. This material was used to test the following plant species for susceptibility to toxins found in the extracts: *Bromus rigidus*, *B. mollis*, *Avena fatua*, *Hordeum leporinum*, *Festuca megalura*, and *Lolium multiflorum*. These grasses are commonly found in annual grasslands adjacent to *Eucalyptus* groves. In addition, *Eucalyptus globulus* was included for comparison.

The bioassay procedure was similar to that described above except that incubation time was dependent upon the species. *Bromus rigidus* was incubated for 48 hr, *Avena*, *Festuca*, *Lolium*, and *B. mollis* were incubated for 72 hr, and the others were incubated for 96 hr. The radicle growth, sample size for the means of each comparison, and Honestly Significant Differences are shown in Table 1. All plants tested were severely inhibited. The order of sensitivity was only roughly correlated with patterns of distribution found in the field. *Hordeum* displayed the greatest tolerance to *Eucalyptus* in the field, often forming pure stands beneath the canopy of *E. globulus* on the edges of the stand, and it was the least inhibited species in these bioassays. The moderate sensitivity of *Bromus rigidus* and *Avena*

Table 1. Radicle growth of annual grassland species subjected to three fold concentration of artificial fog drip from *Eucalyptus globulus*. (All values are means of radicle lengths ± SD for the sample size indicated in parentheses following each mean.)

Species	Control (mm)	Test (mm)	Percent of Control	$HSD_{.05}$ (mm)
Bromus rigidus	24.1 ± 4.4 (30)	11.9 ± 2.6 (30)	49	4.9
Festuca megalura	14.0 ± 2.8 (40)	3.1 ± 1.6 (19)	22	3.2
Bromus mollis	17.7 ± 2.9 (30)	4.1 ± 2.7 (30)	23	3.5
Lolium multiflorum	17.9 ± 5.1 (40)	4.9 ± 1.6 (30)	28	3.0
Avena fatua	31.7 ± 4.6 (27)	12.9 ± 4.5 (25)	41	5.8
Hordeum leporinum	27.6 ± 5.1 (20)	17.4 ± 4.7 (20)	63	5.9
Eucalyptus globulus	15.5 ± 3.8 (72)	7.0 ± 2.7 (73)	45	2.5

and the high sensitivity of *Lolium* and *B. mollis* to these toxins is reflected by their field distributions. However *Festuca* often occurs mixed with *Hordeum* beneath the canopy, a fact that cannot be predicted from these data. In this bioassay series, *E. globulus* was moderately affected, suggesting no adaptation to the compounds found in fog drip. In a less extensive series of bioassays, performed with natural fog drip, *E. globulus* was not inhibited as compared with significant inhibition of *Avena* and *B. rigidus*.

The toxicity of fog drip in soil was also tested by a bioassay technique. In this method a 50-g sample of Milpitas loam soil was substituted for sponges as a bed for germination of *B. rigidus*. Controls were irrigated with 15 ml of distilled water while tests were treated with 15 ml of fog drip. Each was replicated three times with 10 seeds planted in each 500-ml dish. After 48 hr of incubation at 26°C in the dark, radicle growth was measured.

The results (means ± SD) are shown in Table 2. The natural fog drip was the result of a fairly heavy fog and consequently rather dilute. Nevertheless, the reduction to 77% of control was significant at the 5% level by the HSD method. The concentrated artificial fog drip, which inhibited *B. rigidus* on sponges in a simultaneous bioassay to 52% of control, produced inhibition to 65% of control in soil, also significant at the 5% level by the HSD method. These experiments demonstrate that fog drip toxicity is not lost in soil and support the hypothesis of a mechanism of fog as the carrier of an inhibitor of annual herbs.

Detection of toxins. Toxins responsible for the inhibition described above were identified by chromatographic means. Natural fog drip of proven toxicity was injected into a gas chromatograph[4] and compared with injections of distilled water, α-pinene, cineole, and water saturated with these terpenes. These determinations failed to show any terpenes in fog drip. In view of their low water solubility, this is not surprising.

Artificial fog drip was prepared and analyzed by two dimensional descending paper chromatography following the procedures of Smith (1962) for phenolic acids. Eight compounds were clearly separated from this

Table 2. Radicle growth of *Bromus rigidus* in loam treated with natural and artificial fog drip. (N = 30 for all means; radicle lengths are means ± SD.)

Experiment	Control (mm)	Test (mm)	Percent of Control	$HSD_{.05}$ (mm)
Natural fog drip, no concentration	27.4 ± 3.3	21.1 ± 2.8	77	5.1
Artificial fog drip, 3X concentration	27.2 ± 4.4	17.9 ± 3.2	65	4.8

material with butanol: acetic acid: water (6:1:2) followed by 6% acetic acid. There was also a streak from the origin to the acetic acid solvent front representing an unseparated mixture of compounds probably rich in polymers. Of the eight isolated compounds, the characteristics (Rf, color, and UV absorption spectra) of five agreed well with those reported by Hillis (1966) and with those previously determined in this laboratory from *Eucalyptus camaldulensis* (del Moral and Muller, in press). Ellagic acid, a non-toxin, was an important constituent. Chlorogenic, *p*-coumarylquinic, and gentisic acids were consistently present. Each of these is a known toxin. Gallic acid occurred sporadically in trace amounts. Since gallic acid is a major constituent of hydrolyzed leaves (Hillis, 1966), it is likely that it occurs primarily as various polymers and glycosides in fresh living material. The chromatographic streak mentioned above may represent such compounds. Some of these, termed "tannins" or "gallotannins," are well

[4] Aerograph 550 with temperature programmer; SE 30 column; oven temperature = 100°C; injection temperature = 140°C; nitrogen flow = 20 ml/min; hydrogen flow = 20 ml/min.

known for both their toxicity and difficulty of accurate characterization. The presence of all these compounds was confirmed in natural fog drip.

These results show that indeed fog leaches several compounds from living leaves of *E. globulus*. More elaborate and detailed analyses would undoubtedly reveal the presence of several additional types of organic compounds, and probably several mineral compounds.

Discussion. The evidence present in this report indicates that natural fog drip from *Eucalyptus globulus* inhibits the growth of annual grass seedlings in bioassays both on sponges and in soil and suggests that such inhibition occurs under natural conditions. The toxins responsible are also present in fog drip produced artificially in the laboratory. This artificial fog drip was toxic to a variety of grasses and to seedlings of *E. globulus* itself. The fact that *Eucalyptus* seedlings are susceptible to toxins found in fog drip sustains the suggestion of the importance of leaching in the prevention of autotoxicity (Muller, in press).

That natural fog drip from *Eucalyptus globulus* contains several physiologically active components in significant concentrations, including *p*-coumarylquinic, chlorogenic, and gentisic acids, is a fact of great ecological importance. The various chemicals thus liberated from metabolically active tissue fall to the ground with the condensed fog drip. Repeated incidence of fog encourages the accumulation of these toxic compounds in the surface soil (del Moral and Muller, in press) and results in the inhibition of associated plants. In a more diverse flora than that surrounding Santa Barbara groves of *Eucalyptus*, the tree may exert a more subtle influence upon associated vegetation. Species relatively tolerant of such toxins would benefit from the absence of competitors and thrive beneath *Eucalyptus*. Thus in Australia natural groves of *E. globulus* commonly occur with no herbaceous understory and with but a certain few species of shrubs not usually encountered outside the tree's sphere of influence (J. M. Gilbert, personal communication). The diversity of plant communities is influenced by many factors. The reactions of living organisms, when coupled with a gradually changing environment, lead to a vegetational pattern which has spatial and temporal dimensions. This pattern is influenced by mechanisms which alter the homogeneity of the environment which may be regarded as a change in the *alpha* (within individual community) diversity of Whittaker (1965). In this example of a colonizing species in recent contact with introduced herb species, the presence of toxic fog drip lowers the diversity of the *Eucalyptus* stand by creating an environment too severe for some plants that could otherwise exist there.

We must emphasize that toxic fog drip is only one of several mechanisms present in *Euclyptus* species capable of producing herb growth inhibition. Leaf litter possesses many toxic phenolic acids which are leached in great quantities by rain. Terpenes volatilized from leaves are adsorbed by soil colloids. Soil in this condition is highly inhibitory to germinating seedlings (Muller and del Moral, 1966; del Moral and Muller, in press). However, the evidence presented here strongly suggests that in this species toxin transfer by fog drip alone is capable of severely inhibiting the growth of annual herbs. We suggest that fog drip from such species as *Sequoia sempervirens*, *Pseudotsuga menziesii*, and *Cupressus macrocarpa* in California may be partially responsible for the striking paucity of herbs in forests dominated by these species.

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