tabolites has been reported to be the mechanism of biological control by many *Trichoderma*, *Gliocladium* species, bacteria and actinomycetes. Isonitrite was isolated from *T. hamatum*[16,17]; T-2 toxin from *T. lignorum*[18] and gliotoxin and gliovirin from culture filtrate of *G. virens*[19]. As seen in the present study, it is possible that purified compounds contain some antifungal elements and hence, TI, Tpk and Tk showed inhibition of mycelial growth of *N. indica*. It is further clear from fractionation on TLC plates that isolates TI, Gv, FP-VII, Act-V and Bact-II produced atleast three antifungal compounds.

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ACKNOWLEDGEMENTS. We thank Head, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi for providing necessary facilities and valuable suggestions. G. A. M. A. is also thankful to the Government of Egypt for granting him fellowship to conduct the present research work as part of his Ph D programme.

Received 6 October 1997; revised accepted 3 October 1998

Chemical stimulation of germination and membrane fluidity change in secondarily dormant cucumber seeds

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Relative efficacy of various dormancy breaking chemicals is known to relate to their physical/chemical properties. Acetaldehyde required a much shorter treatment time to stimulate the germination of secondarily dormant cucumber (*Cucumis sativus* L.) seeds than ethanol and other less lipophilic compounds. T_{50} (exposure time required for 50% germination) was inversely correlated to the K_o/w (octanol–water partition coefficients) of the chemicals applied. Lipid bilayer fluidity in microsomal membranes of chemically-treated seeds was greater than in untreated control seeds imbibed in water for an equal time period. There was a significantly positive correlation between T_{50} and fluorescence anisotropy of the outer lipid monolayer in seed microsomal membranes, which indicated that membrane fluidity could be a significant event during the process of chemically-induced germination of secondarily dormant cucumber seeds.

SEED dormancy is broken by a number of organic compounds including alcohols, aldehydes and ketones[1–4]. Besides having an applied potential, they serve as molecular probes to explore the mechanisms involved in the transition from developmental arrest to growth[5]. While a number of workers[6–8] proposed a metabolic role for alcohols and related substances in stimulation of germination, others[9–11] suggested that they act at the membrane level. Interestingly, dormancy breaking activity of a large number of substances was shown to be correlated with the lipophilicity of the applied chemical[3,5]. However, all these studies focussed on the effects of chemicals on primary dormancy. Furthermore, very limited studies have been undertaken so far to correlate the effects of nonhormonal chemicals with the membrane functions[4].

Secondary seed dormancy in cucumber is known to be released by acetone, ethanol and a few other organic compounds with a concomitant change in the permeability of the cell membranes[12]. The aim of the present investigation was to determine (i) whether the dormancy-breaking activity of certain nonhormonal chemicals in cucumber is a function of their lipophilicity, and (ii) whether a change in fluidity, if any, in seed cellular membranes occurs during the chemical-induced transition from dormancy to germination in secondarily dormant cucumber seeds.

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Seeds of cucumber (*Cucumis sativus* L. cv. Poinsett 76) were purchased from Indo-American Exports, Bangalore, India. Dormancy was induced by exposing non-dormant seeds (germination ~ 95% at 20°C in darkness) to intermittent far-red light (7.5 µmols m^{-2} s^{-1}) (ref. 13) while imbibing in – 1.8 MPa polyethylene glycol solution in darkness at 20 ± 1°C. Secondarily dormant seeds thus obtained (germination ~ 10% at 20°C in darkness; viability ~ 95% in tetrazolium tests[14] were immersed for various durations in acetaldehyde, *n*-propanol, acetone, ethanol and methanol in glass-stoppered flasks at 10°C. Besides offering a reasonable K_o/w range (octanol–water partition coefficients)[15], the selected chemicals showed no visible toxic effects on germination and subsequent seedling growth, at least for the treatment duration and temperature tested. After the treatment, the seeds were air-dried for 24 h and then used in germination and other studies.

All germination tests were conducted with 25 seeds per replicate and 4 replicates per treatment. Seeds were allowed to imbibe on water-saturated filter paper circles for 3 days at 20 ± 1°C in darkness. The criterion for germination was the emergence of the radicle through the testa. The germination experiments were repeated at least twice. The average values for percent germination were arcsin transformed before statistical analysis.

Microsomal membranes were isolated from 18 h water-imbibed dormant (control)/chemically-treated seeds following Hodges and Mills[16] and Wheeler and Boss[17]. This period was selected because splitting of the perisperm–endosperm envelope, the first visible germination event in cucumber seed, began between 18 and 24 h of dark imbibition in water at 20°C in the chemically-treated seeds. Changes in the structure and functions of membranes, if any; after this time period could be an admixture of germination/seedling biochemistry not highly relevant to the transition from dormancy to germination. Decoated seeds (testa removed) were homogenized in 50 mM Tris-MES (pH 7.2) containing 0.25 mM sucrose, 3 mM ethylenediamine tetra-acetic acid (EDTA), 2.5 mM dithiothreitol (DTT), 2 mM phenylmethylsulphonyl fluoride (PMSF) and 0.6% insoluble polyvinylpyrrolidone (PVP) in a prechilled pestle-mortar. The homogenate was filtered through a nylon mesh and centrifuged at 10,000 g for 10 min at 0°C in a refrigerated centrifuge with a fixed angle rotor (Kubota 6900, Japan). The supernatant was recentrifuged at 36,000 g for 45 min at 0°C and the resulting pellet (microsomal fraction) was suspended in a medium containing 5 mM potassium phosphate (pH 7.8), 0.25 M sucrose and 50 mM EDTA. The absorbance of the membrane suspension was adjusted to 0.05 absorbance unit at the corresponding excitation peaks each for 1,6-diphenyl 1,3,5-hexatriene (DPH), and its derivatives DPH-propionic acid (DPH-PA) and trimethylammonium-DPH (TMA-DPH) to standardize light scattering[18].

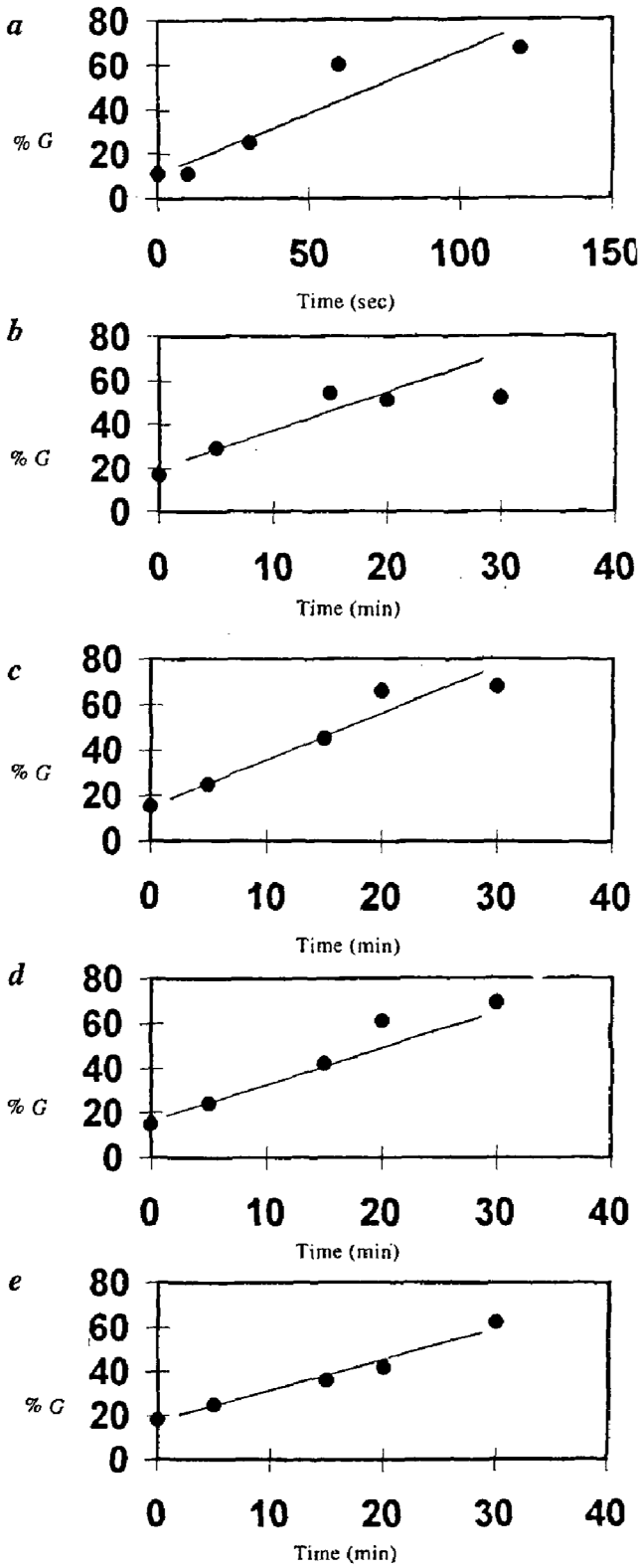

Figure 1. Effect of acetaldehyde (*a*), *n*-propanol (*b*), acetone (*c*), ethanol (*d*), and methanol (*e*) on germination (expressed as % G) of secondarily dormant cucumber seeds.

Fifty microlitres of 2 mM DPH, DPH-PA and TMA-DPH each in dimethylsulphoxide were added in 25 ml vigorously stirring suspension medium. Twenty five

Table 1. Fluorescence anisotropy of DPH-, DPH-PA-, and TMA-DPH-labelled microsomal membranes from 18 h water-imbibed dormant (control) and chemically-treated seeds

Chemical	Fluorescence anisotropy		
	DPH	DPH–PA	TMA–DPH
Dormant (control)	0.266 ± 0.008	0.427 ± 0.006	0.401 ± 0.006
Acetaldehyde	0.249 ± 0.004	0.400 ± 0.003	0.385 ± 0.006
n-propanol	0.252 ± 0.007	0.408 ± 0.002	0.395 ± 0.002
Acetone	0.252 ± 0.003	0.403 ± 0.005	0.382 ± 0.006
Ethanol	0.248 ± 0.003	0.408 ± 0.004	0.389 ± 0.003
Methanol	0.267 ± 0.007	0.413 ± 0.005	0.398 ± 0.004
r (T_{50} vs anisotropy)	0.57^{ns}	0.89*	0.61^{ns}

*Significant; ns, not significant, at $p = 0.05$.

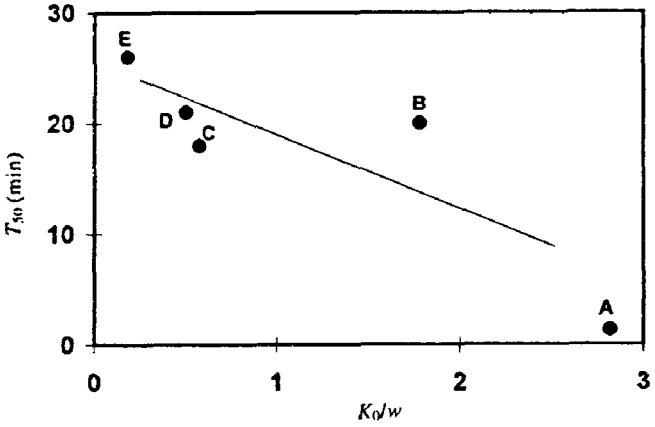

Figure 2. Relationship between K_o/w (octanol–water partition coefficients) and T_{50} (time required to stimulate 50% germination); acetaldehyde (*A*), *n*-propanol (*B*), acetone (*C*), ethanol (*D*), and methanol (*E*).

microlitres for each probe were added separately to 5.0 ml membrane suspension. Labelling was performed at 37°C for 30 min. Fluorescence polarization measurements were carried out with LS 30 luminescence spectrometer (Perkin-Elmer, UK). The samples at 20°C were excited with vertically polarized light at 350 nm (DPH) 354 nm (DPH-PA) and 355 nm (TMA-DPH) and, vertically and horizontally polarized light emissions were read at 452 nm (DPH) and 430 nm (DPH-PA and TMA-DPH). Anisotropy values were calculated from the degree of polarization in accordance with Shinitzky and Barenholz[19]. Acetaldehyde required relatively much shorter exposure time to evoke germination level equal to other chemicals (Figure 1). Among other chemicals, a 20 min exposure to acetone and ethanol induced much higher germination than an equal exposure to *n*-propanol and methanol. Treating seeds more than 30 min with *n*-propanol and methanol, but not with acetone and ethanol, reduced percent germination possibly on account of toxicity (data not given). Because the maximum germination inducible with *n*-propanol was relatively lower than that obtained with other chemicals, T_{50} (exposure time required for 50% germination) for each chemical was determined from a regression equation (Figure 1) and was plotted against K_o/w (an index of lipophilicity) for each chemical for lipophilicity – activity comparisons (Figure 2). Acetaldehyde, which had the highest lipophilicity among the chemicals tested, required lowest exposure time to induce 50% germination, whereas methanol having the lowest lipophilicity showed highest T_{50}. Cohn[3], who used primarily dormant red rice seeds as a model system, showed the efficacy of dormancy-breaking compounds applied in aqueous phase to be an inverse function of their lipophilicity. In the present work with secondarily dormant cucumber seeds also a negative correlation was found between K_o/w and dormancy-breaking activity of the tested chemicals applied in nonaqueous phase.

Besides alcohols, several dormancy-breaking compounds such as ethyl ether, chloroform, acetone and others are known to affect the membranes[4,10,11]. The fluorescence polarization has proven to be one of the most sensitive, reproducible and convenient means of probing the fluidity and organization of membranes[20]. Comparison of fluorescence anisotropy, as monitored by DPH, which is known to partition uniformly in the lipid bilayer thus giving an overall reflection of the membrane status[18,19], indicated an increase in the fluidity of membranes with all the chemicals except methanol (Table 1). This suggested that the lipid bilayer in microsomal membranes in dormant seeds was relatively more rigid than those from treated seeds. However, because the membranes isolated from the dry dormant (control) and chemically-treated seeds showed little difference in fluorescence anisotropy values (data not shown), presumably the change occurred later in the germination.

DPH-derivatives have been used as probes to monitor alcohol-induced membrane perturbations. DPH-PA, an

anionic derivative, distributes primarily in the outer lipid monolayer, whereas, the cationic derivative TMA-DPH preferentially localizes in the inner lipid monolayer[21]. While the fluidity of the outer leaflet, as reported by DPH-PA, as well as that of the inner leaflet, as probed by TMA-DPH, were found to increase during the chemically-induced transition from dormancy to germination, it was only in case of the outer lipid monolayer that a significant correlation was observed between the fluorescence anisotropy value and T_{50} for each chemical (Table 1). However, it would be rather premature to suggest a causative relationship between membrane fluidity and dormancy breaking and the observed relationship could be described, with the data in hand, as correlative at best.

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ACKNOWLEDGEMENTS. We are grateful to the Department of Science and Technology, New Delhi for financial support of the Project SP/SO/A-27/94.

Received 18 April 1998; revised accepted 21 September 1998.

Canopy effect on the dry matter allocation in different components of evergreen and non-evergreen forb species of Kumaun Himalaya

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Two forb species, *Artemisia vulgaris* and *Arisaema concinuum* occurring in both open and closed canopy forests have been selected for the study of resource allocation pattern under different light, moisture content and pH conditions to individuals of both species were randomly selected and harvested at peak of their growth. The distribution of dry mass in the underground, stem, leaves and in reproductive components were determined. In open canopy, both forb species had relatively greater dry mass invested in their reproductive activity than that of individuals occurring at the closed canopy site. However, the allocation of dry mass to supporting and assimilatory system of plants were adversely affected by direct sunlight, low moisture content, pH of soil and less fertile soil character of open canopy site than at close-canopy site. Regression analysis showed strong correlation between reproductive organ biomass and total biomass in individuals at the open canopy site.

THE knowledge of dry matter production by herbs is useful in studies of photosynthetic capture and dry matter production of the herb layer. For the tree seedlings, Madgwick *et al.*[1] emphasized that the relative distribution of photosynthates to leaves, stems and roots of young tree seedlings, is related to the ultimate biomass production of the trees.

Canopy affects the productivity and species composition of understorey grasslands[2–4]. The pattern of biomass distribution to the various plant parts differs between individuals of the same species growing in different habitats[5–8].

The distribution of biomass among various vegetative plant organs depends, to a large degree on the nature of the limiting resource(s), i.e. nutrients, water, light, etc.[9,10]. Plant species from habitats that are less mature or more highly disturbed tend to allocate a great proportion of total biomass to reproductive parts when compared with plants of more mature or relatively less disturbed areas[9]. Variation in allocation and morphology within individual species should follow patterns predicted for communities[11].

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