Use of 2,4-D as an Inhibitor of Germination in Routine Examinations of Beans for Seed-Borne Infection[1]

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During the examination of samples of bean seed, *Phaseolus vulgaris* L., difficulties were experienced in determining the percentage of seeds infected with *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. The method of examination used was that described by Groves and Skolko (*2*). This method proved to be quite suitable for seeds of plants with hypogeal cotyledons, but somewhat unsatisfactory for bean seeds. With beans, the agar became cracked and displaced, and the hypocotyls elongated to such an extent that the cotyledons became displaced from their original positions on the agar. At the end of the period of incubation, it was difficult to ascertain from which of the seeds the pathogen had developed (Fig. 1, lower row).

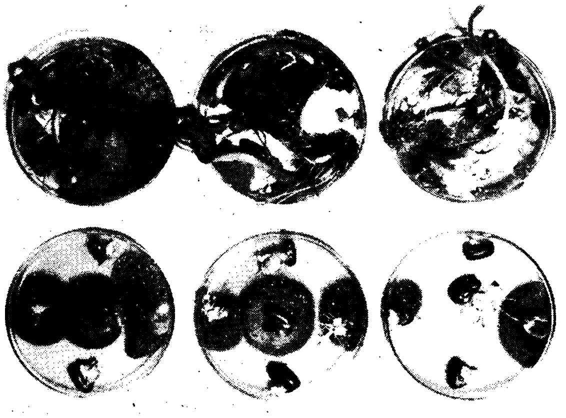

FIG. 1. Surface-sterilized bean seeds incubated 14 days at 20–25° C on malt extract agar. Upper row, unmodified medium; lower row, medium containing 50 ppm of sodium 2,4-dichlorophenoxyacetate.

Attempts were made to inhibit seed germination coincident with adequate development of the pathogen. Incubation at a reduced temperature was only partially effective, but chemical inhibition of germination proved to be very effective. Hamner, Moulton, and Tukey (*3*) have shown that traces of 2,4-dichlorophenoxyacetic acid in soil can affect the germination and growth of many seeds, including beans. Accordingly, additions were made to the culture medium of either this chemical or its sodium salt, sodium 2,4-dichlorophenoxyacetate. The sodium salt resulted in less hydrolysis of the agar during autoclaving and therefore proved to be the more satisfactory of the two chemicals. When it was included in the nutrient medium at a concentration equivalent to 25–300 ppm of the acid, germination of the bean seeds was strongly inhibited.

It was found that low concentrations of the chemical would inhibit germination without causing any observable retardation of the growth of *C. lindemuthianum*. Bever and Slife (*1*) reported retardation or killing of *Pythium debaryanum*, *Gibberella zeae*, and *Helminthosporium victoriae* by 2,4-D in concentrations between 250 and 2000 ppm of the active acid. In the present study some retardation of *C. lindemuthianum* was found at concentrations above 100 ppm, but none at lower concentrations. It was also evident that there were strains of this pathogen that differed from one another in respect to their tolerance for higher concentrations of 2,4-D.

The results of repeated tests appear to warrant the use of 50 ppm, acid equivalent, of sodium 2,4-dichlorophenoxyacetate in culture media used for routine examination of bean seeds for infection with *C. lindemuthianum*. A satisfactory medium has the following composition: malt extract 20 g, agar 15 g, added to 1000 ml of solution containing 50 ppm, acid equivalent, of sodium 2,4-dichlorophenoxyacetate. This medium has a pH of 4.7 as determined by means of a Beckman glass electrode pH meter.

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Relation between Band Slicks at the Surface and Internal Waves in the Sea[1]

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Band slicks on the sea surface like those shown in Fig. 1 are commonly seen along the shore when the wind is a light breeze. Dietz and La Fond (*1*) of the Naval Electronics Laboratory in San Diego, California, have observed such slicks in coastal waters of California, Australia, and Samoa, around the Marquesas Islands, and near the Antarctic ice pack. Woodcock and Wyman (*6*) have observed and photographed similar "bands of light and dark appearance" in the Gulf of Panama. They attrib-

[1] Contributions from New Series, No. 448, Scripps Institution of Oceanography.

[2] The author is grateful to the Naval Electronics Laboratory, San Diego, for the loan of equipment.

uted the effect to a systematic variation in the pattern of small waves on the sea surface associated with a corresponding variation in wind speed, which they explained in terms of roll vortices caused by convection in the air or in terms of an internal wave in the air caused by wind shear near the sea surface. They observed the effect at all wind speeds up to 7.5 m per sec and described the bands as oriented with their long axes roughly parallel to the wind.

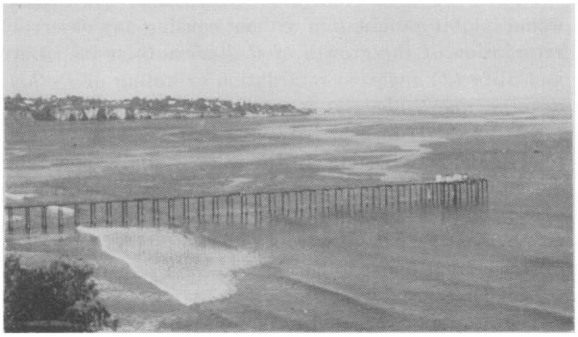

FIG. 1. Typical band slicks.

Dietz and La Fond (*1*) have found that the primary mechanism controlling the formation of slicks in bands is a film of some substance at the sea surface which lowers the surface tension in the bands where ripples are absent, the role of the wind in this connection being merely to generate ripples which reveal the peculiar disposition of the surface film. A secondary mechanism must be sought to explain why the surface film is unevenly distributed. Detailed observations by the writer have revealed that at least two such distributive mechanisms are in operation: one, which dominates when the wind is absent or light, is associated with internal waves in a shallow thermocline under the sea surface; the other, which dominates at higher wind velocities, is controlled by processes in the air, such as Woodcock and Wyman (*6*) describe. In order to keep the two cases distinct, slicks formed by the first process will in this report be called internal wave slicks, and those formed by the second will be called wind slicks. Internal wave slicks form over the troughs of internal waves, travel with the waves, and are oriented with their long axes parallel to the wave troughs without respect to wind direction. Wind slicks, on the other hand, are independent of internal waves in the sea and are oriented in an approximately downwind direction. The transition from one regime to the other occurs roughly when the wind speed is greater than 3.5 m per sec, i.e., the speed necessary to straighten out light flags and to keep leaves and twigs in constant motion.

More work will be needed to confirm the relationship between band slicks and internal waves, but the evidence so far is convincing enough to warrant a preliminary report. The evidence presented is of two sorts: first, in the series of simultaneous bathythermograph and slick observations shown in Fig. 2, the slicks coincide in every case with depression of the 60° F isotherm and the rippled zones coincide with elevation of the isotherm; and second, when, as in Table 1, the most significant of the

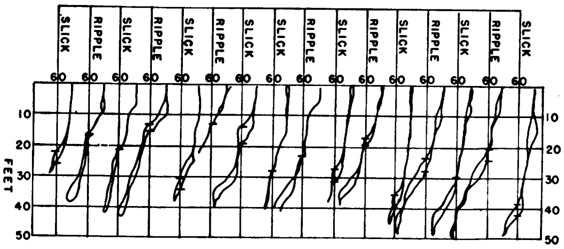

FIG. 2. A series of bathythermograms taken in rapid succession from a moving ship together with simultaneous observations of internal wave slicks, showing the elevations and depressions of the 60° F isotherm in relation to the presence of ripples or slicks at the surface.

observed characteristics of internal wave slicks are arrayed against the oceanic phenomena to which they may readily be attributed, it is seen that short period internal waves give the simplest explanation of the observations, since this phenomenon by itself can account for all the listed characteristics, whereas, of the remaining phenomena in the table, no less than four must be supposed to be operating in concert to account for the complete list.

Before discussing Table 1 in detail, a brief résumé of the known properties of internal waves will be given, to-

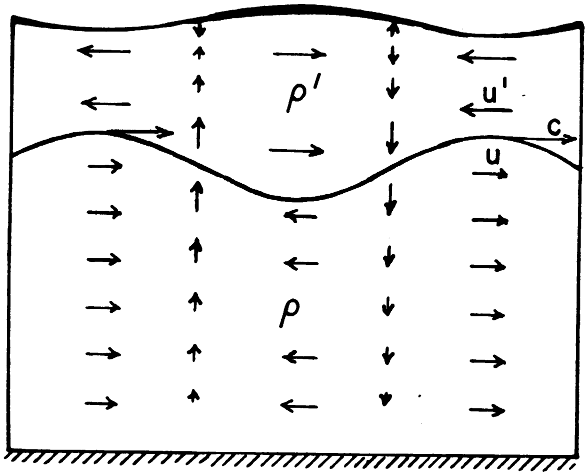

FIG. 3. Schematic diagram of an internal wave in two layers of water of nearly equal densities, ρ' and ρ, where the wavelength is large compared to the thickness of either layer. Shown are the phase velocity, c, the horizontal particle velocities in the upper and lower layers, u' and u, and the associated vertical velocities. The surface layer is alternately thickened and thinned because of the convergence and divergence of u'. This effect, as well as the amplitude of the wave at the surface, has been much exaggerated for purposes of illustration. (After Sverdrup.)

gether with a suggested mechanism to account for the uneven disposition of surface-active materials. Fig. 3 is a diagrammatic representation of an internal wave in the thermocline between two layers of water of nearly equal density, where the wavelength is great compared to the thickness of either layer. Internal waves of this type are described by Lamb (*3*), Sverdrup (*4*), Ufford (*5*), Haurwitz (*2*), and others. The fundamental feature causing internal waves to behave differently from ordinary surface waves is the presence over the wave of a fluid nearly as dense as that below. Because of this the potential energy associated with a given deformation is much reduced, while the inertia of the system as a whole is increased. Consequently, when an internal wave is compared to an ordinary surface wave of similar length, it is found that the speed of propagation, or phase velocity, of the internal wave is diminished in the ratio

TABLE 1

RELATION OF OCEANIC PHENOMENA TO OBSERVED CHARACTERISTICS OF BAND SLICKS

Oceanic phenomenon	Characteristics of band slicks							
	Banding (1)	$c \approx 25$ cm/sec (2)	u' oscillates (3)	$u'_{\max} \rightarrow c$ (4)	$T \approx 1/3$ hr (5)	Persistence (6)	Refraction (7)	Independence of wind (8)
Transport by wind or current			X			X		
Transport by surface waves	X*		X		X			
Internal waves in the air							X	X
Roll vortices in the air							X	X
Surface waves, swell, seiche		X		X				
Tides, as waves	X	X			X	X		
Internal waves in water	G†	G	G	G	G	G	G	G

* X = bad fit.
† G = good fit.

$\{(1-s)/(1+s)\}^{1/2}$, where s is the ratio of the density of the upper to that of the lower fluid. For the usual case in the sea, this factor is of the order of 10^{-2}. The orbital motion of individual water particles for the case diagrammed in Fig. 3 is in opposite sense in the two layers. In the upper layer, the orbits are ellipses whose horizontal axes are uniform throughout the layer and whose vertical axes vary inversely with the distance above the thermocline. Thus the horizontal component of particle motion is the same at all depths in the upper layer, being in the direction of wave propagation over the trough of the wave and in the opposite direction over the crest.

The vertical component of particle motion, on the other hand, is comparable to the horizontal component only at the thermocline, decreasing rapidly above this level and vanishing at a depth of a centimeter or so below the free surface. At still higher levels the vertical motion is reversed, so that, at the free surface, there exists a wave of very low amplitude exactly out of phase with the wave at the thermocline. It can be seen in Fig. 3 that the horizontal particle velocities are convergent wherever the thermocline is descending and divergent wherever the thermocline is rising. The convergence is most intense halfway between crest and trough, but its cumulative effect is greatest over the trough of the lower wave. Thus, if the water above the thermocline were imagined divided into a number of layers, each layer would be thickest over the trough of the wave and thinnest over the crest. This effect, acting on a surface film of initially uniform thickness, would distort it into alternate bands of greater thickness over the wave trough and less thickness over the wave crest. Thus a slick would be formed by temporary thickening of the surface film in a given area during the time the trough of an internal wave was passing beneath it. The zone of thickening, rather than the material itself, would thus progress with the internal wave. The effect of a wind-driven current on this mechanism would depend on the size of its cross-slick component. For small values, it would merely displace the slick with reference to the trough. At critical values it would cause the slick material to drift with the wave so that the material in a given slick would remain the same. At values greater than the critical, the wind-driven current would completely dominate the slick material and would then produce wind slicks nearly independent of internal waves, in the manner described by Woodcock and Wyman (*6*).

The characteristics of wave slicks listed in Table 1 are based on the following observations:

1. *Banding.* The slicks are about 30 m wide and often many kilometers long. They lie in roughly parallel deployment, as bands of clouds sometimes do, separated by zones of ripples about 300 m wide. Ufford (*5*) found internal waves off San Diego of from 100- to 400-m length between crests.

2. *Slick velocity, $c \approx 25$ cm/sec.* Time-motion studies show that internal wave slicks often move in a direction perpendicular to their long axes at a uniform and steady speed of about ½ knot. This velocity is relatively greater in deep water. Ufford (*5*) found the phase velocity of internal waves to be from 0.07 to 0.68 knots.

3. *Particle velocity, u', oscillates.* Floating objects execute oscillatory motion perpendicular to the long axis of a passing slick. They are drawn into the oncoming slick, held in the slick for a time, and finally expelled to the rear, nearly resuming their initial position.

4. *Maximum horizontal particle velocity, $u'_{\max} \rightarrow c$.* Objects, while floating in a slick, advance for a time at a speed significantly comparable to the phase velocity. Such high particle velocities are characteristic of internal waves.

5. *Period, $T \approx 1/3$ hr.* Internal wave slicks usually move past a point at intervals of from 15 to 30 min. Ufford found internal waves with periods from 9 to 136 min.

6. *Persistence.* Slicks persist in spite of horizontal eddy diffusion. This shows that they are continually maintained by a disturbance moving with the slick.

7. *Refraction.* Internal wave slicks, in water less than 20 m deep, are oriented approximately parallel to the bottom contours. Thus upon approaching submarine canyons, for example, they are bowed into the canyons in the manner of long period swell. The speed of internal waves depends partially on the water depth below the thermocline. For the case described above the speed is given by:

$$c^2 = g\,\frac{h\,h'}{h+h'}\,\frac{\varrho-\varrho'}{\varrho}$$

where h refers to the thickness of the layers ϱ refers to their densities and the primes refer to the upper layer.

8. *Independence of wind.* In the absence of wind, band slicks are not visible because there are no ripples, but floating objects are often observed to be marshalled in long rows at the sea surface. As soon as the wind arises it is seen that slicks form along these rows. At wind velocities less than about 3.5 m per sec the orientation of the slicks is independent of wind direction.

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β-3-Thienylalanine, an Antiphenylalanine in the Protein-depleted Rat[1]

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Most studies of the effects of amino acid antagonists on mammalian metabolism have been made with young growing rats (*1, 3*). Since the protein-depleted rat regains weight rapidly on a synthetic diet containing all the essential amino acids (*2*), it seemed to us that this uniformly selected animal should be useful for the study of amino acid antagonists. At this time we wish to report the effect of β-3-thienylalanine (β-3-TA) on the recovery of the protein-depleted rat.

TABLE 1

COMPOSITION OF BASAL (MEA) DIET

	%		%
Dextrin	61.14*	Essential amino acids†	3.93
Corn Oil	4.00	Nonessential amino acids‡	4.93
Fiber	5.00	Vitamins in dextrin	1.00
O-M Salt Mix§	4.00	Water	16.00

* Various addenda were made by reducing the content of dextrin by a corresponding amount.

† Composition of the essential amino acids in this mixture was: L-histidine-HCl, 4.91%; DL-isoleucine, 20.69%; L-leucine, 12.29%; L-lysine-HCl, 13.49%; DL-phenylalanine, 7.63%; DL-threonine, 14.58%; DL-tryptophane, 2.47%; DL-valine, 17.30%; DL-methionine, 6.62%.

‡ Composition of the nonessential amino acids in this mixture was: DL-alanine, 11.71%; L-arginine-HCl, 10.37%; DL-aspartic acid, 13.17%; L-cystine, 0.75%; L-glutamic acid, 49.57%; glycine, 1.06%; and L-tyrosine, 13.37%.

§ Osborne and Mendel salt mix.

[1] This work was supported in part by research contracts with the Office of Naval Research.

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Albino male rats of the Sprague-Dawley strain with initial weights varying from 211 g to 227 g were selected. They were depleted of protein according to procedures previously described (*5*), and selected for the experiment when their weight loss was between 26% and 35%. During the first two days of the experimental period all animals in individual cages were offered 15 g of the basal diet containing the minimum amounts of the essential amino acids required for optimum restoration of lost weight (*4*). The composition of this diet, designated MEA, is given in Table 1. After two days on the MEA diet, 12 rats were divided into four groups of three animals each. Each animal in the four groups received the dietary treatment as outlined in Table 2.

TABLE 2

DIETARY TREATMENT OF EACH GROUP OF RATS

Group 1	MEA for 10 days.
Group 2	MEA for 2 days; MEA plus 90 mg phenylalanine for 8 days.
Group 3	MEA for 2 days; MEA plus 50 mg β-3-TA for 1 day; MEA plus 25 mg β-3-TA for 2 days; MEA plus 25 mg β-3-TA and 90 mg phenylalanine for 5 days.
Group 4	MEA for 2 days; MEA plus 200 mg β-3-TA for 2 days; MEA for 2 days; MEA plus 25 mg β-3-TA for 4 days.

The average changes in body weight of each group of animals are plotted in Fig. 1. It may be seen that the animals of groups 1 and 2 gained equal amounts of body weight, indicating that twice the level of phenylalanine did not alter the weight restoration.

The addition of 50 mg of β-3-thienylalanine to the diet resulted in a loss of weight and a decrease in appetite. All animals continued to lose weight when the amount of β-3-TA was reduced to 25 mg per 15 g of diet. When 90 mg of phenylalanine was added with 25 mg β-3-TA, all

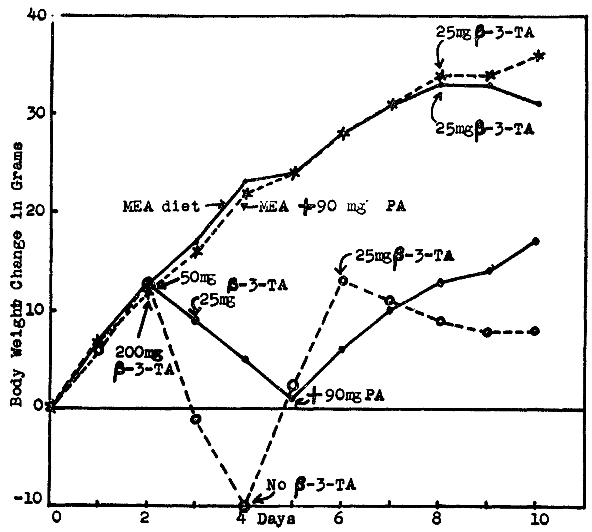

FIG. 1. Curves showing the average changes in body weight of four groups of animals, illustrating the antiphenylalanine properties of β-3-thienylalanine (β-3-TA) in the protein-depleted rat.