Botany. — *An Analysis of Phototropism in Dicotyledons.* By J. VAN OVERBEEK. (Communicated by Prof. F. A. F. C. WENT.)

(Communicated at the meeting of December 17, 1932).

A hundred years ago DE CANDOLLE (1) tried to account for the phototropic curvature by the fact that plants, when growing in the dark, elongated more rapidly than when growing in the light. When a plant is illuminated from one side only, the illuminated side will get more light than the shaded side. The latter will then grow quicker than the illuminated side and consequently the plant will show a curvature towards the light.

We find the same principle later in BLAAUW's theory of the light-growth-reaction. BLAAUW (2) has studied the growth-reaction in relation to various quantities of light. He found that the shaded side of the hypocotyl of *Helianthus* (when illuminated from one side) receives several times less light than the lighted side. He supposed that the difference between the light-growth-reactions on both sides is the cause of the phototropic curvature (3).

Several years later, as the importance of the growth-substance became increasingly evident, F. W. WENT (4) worked out a theory that is based upon the unequal distribution of the growth-substance. From the tip of a seedling of *Avena*, a stream of growth-substance travels downward. In the dark the growth-substance is distributed equally. On illumination of one side, however, the stream of growth-substance flows to the shaded side. Thus this side receives much more growth-substance than the illuminated side. According to WENT's theory this process causes the phototropic curvature.

The relation between growth-substance and phototropism has been studied up to this time, almost exclusively with seedlings of Monocotyledons. In this paper an attempt is made to investigate this relation in Dicotyledons.

As a great amount of plant material was needed each day, the use of seedlings as experimental objects was obvious. At the beginning those of *Lepidium sativum* and *Raphanus sativus* seemed well adapted to our work. *Lepidium*, however, soon turned out to be too small and therefore difficult to handle. *Raphanus* has the advantage of its larger size and of the availability of "pure lines". These are the reasons why I have chiefly worked with *Raphanus*. The pure race I worked with, was named "IJskegels" (meaning in Dutch: icicles; long and white radish).

The seeds were sown in earth in a hothouse. The average temperature was 22° C., but often oscillated greatly, mainly on days with a clear sky. Five or six days after sowing the seedlings were ready for the experiments. The plants then had a length of about 4 cm. and large, leaf-like cotyledons. At the tip of the hypocotyl, between the stalks of the cotyledons, the epicotyl (the young stem above the cotyledons) was scarcely visible.

Growth-substance may be extracted from the tip and especially from the cotyledons in appreciable quantities. The quantity of growth-substance extracted from one single cotyledon by a block of agar of $2 \times 2 \times 0.9$ mm. is shown in table 1. This quantity is expressed in grades of curvature of a coleoptile of *Avena*, on which this block of agar (containing growth-substance) is placed asymmetrically.

TABLE 1.

Quantities of growth-substance extracted from the cotyledons of *Raphanus*. 3 blocks of pure agar were placed successively on the same cotyledon. Extraction time: 3 hours for each block. Mean value for 12 cotyledons.

first 3 hours	26°
second 3 „	30°
third 3 „	10°

In accordance with the fact that the cotyledons produce an appreciable amount of growth-substance, the elongation of the seedlings, *grown* in the hothouse (but *observed* in the darkroom under constant conditions) show an important and permanent decrease in growth velocity when the cotyledons are removed. In that case the growth never stops entirely, however. The growth ceases practically entirely and permanently, when the whole tip is severed. The growth velocity of the 5 or 6 days-old (after sowing) intact seedlings, under the conditions mentioned above is about 1—1½ mm./hour and remains pretty nearly constant during the 5—6 days after sowing.

When we put hothouse-grown seedlings in the dark for a certain time, we see that the quantity of growth-substance, extracted from the cotyledons, decreases. (Table 2.)

TABLE 2.

Quantities of growth-substance extracted from cotyledons of hothouse seedlings of *Lepidium*. 2 series in the daylight and 2 series kept in the dark, 15 hours before the beginning of the experiment. Cotyledons removed from the seedlings at the beginning of the experiment. Extraction time 3½ hours. Mean of 12 cotyledons.

Light	Dark
14 ± 3.6	0
14.2 ± 2.8	2 ± 2

This summary of some general properties of the seedlings seems necessary, for a better understanding of the phototropic reactions.

In the analysis of the phototropic curvature of the hypocotyl, it is of great importance to know how the growth-substance is distributed in the hypocotyl between the illuminated and shaded sides. The exceedingly embracing work of F. W. WENT has also paved the way to further investigation in this direction.

Not only is the distribution of growth-substance in the hypocotyl of importance for the analysis of the phototropic curvature, but also the sensitivity to growth-substance of the growing cells on both sides of the hypocotyl, illuminated from one side.

Therefore the following questions had to be considered more closely:

I. The influence of light on the sensitivity to growth-substance of the cells of the hypocotyl.

II. The distribution of growth-substance in the hypocotyl.
   i. The distribution after one-sided exposure.
   ii. The way in which this distribution originates.

I. *The sensitivity of the cells to growth-substance.*

The hothouse-grown seedlings of *Raphanus* were planted in small troughs of zinc, one day before the beginning of the experiments. Then the plants were placed on the disc of a clinostat revolving in a horizontal plane. The plants were thus exposed to the light on all sides and therefore grew straight. About 4 hours before the beginning of an experiment the cotyledons were removed. Small blocks of agar, containing growth-substance, were placed on the top of the resulting stump. The blocks were placed on one of the remaining parts of the stalks of the cotyledons. (Scheme fig. 1.) Dr. A. J. HAAGEN SMIT had the kindness to provide me with growth-substance (auxin) isolated from urin (5). The plants remained under a bell-jar to prevent the blocks of agar from drying out. One series of the prepared plants remained on the clinostat in the light; another series of plants was placed in the dark (under a black glass cover or in a darkroom with the same temperature). For two or three hours (the duration of an experiment) the temperature has to be constant. This was easily realized on days with a cloudy sky.

As the placing of growth-substance on one side of a hypocotyl causes more longitudinal-growth on this side than on the other, the result is a curvature of the hypocotyl. The curved hypocotyls were photographed and the photographs were measured in order to determine the amount of curvature.

As shown in table 3 and fig. 1, *the curvature is much greater in the dark than in the light, although in both cases the concentration of the growth-substance was exactly the same.*

TABLE 3.

Curvature of hypocotyls of *Raphanus* caused by the asymmetrical placing of auxin on the top. In all-sided light and in the dark. Sum of 2×7 experiments (113 hypocotyls). Time: 3 hours. Concentration of the auxin 30°.

All-sided light	Dark
39.6 (24 %)	123 (76 %)

This difference in curvature may be attributed to the following causes:

a. The intensity of the stream of growth-substance is inhibited by the light and therefore the exposed growing parts of the hypocotyl obtain less growth-substance than those in the dark.

b. With the same quantity of growth-substance, the cells in the light grow less than those in the dark. The cells in the dark are therefore more sensitive to growth-substance than those in the light.

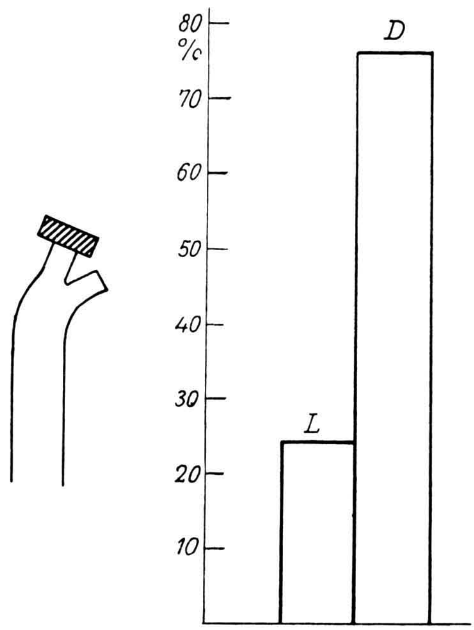

Fig. 1. Relative curvatures of hypocotyls of *Raphanus* which were one-sidedly provided with auxin. On the left a scheme of the experiment: a hypocotyl with on its top a block of agar containing growth-substance. The height (ordinate) of the column *L* represents the size of the curvature in all-round light, that of the column *D* the size of the curvature in the dark.

Ad a. In order to comparate the stream of the growth-substance in the light and in the dark, I cut cylinders from hypocotyls, which were placed on blocks of pure agar. On top of each cylinder was placed a block of agar containing growth-substance. One series of these cylinders was put in the dark and an other series remained in the light. At the beginning of these experiments daylight was also used, as in the experiments of fig. 1, but later on I used only artificial light. The result, however, was the same in both cases. After a certain transportation time the lower blocks were analysed for their quantity of growth-substance. This analysis was performed in the usual way, with etiolated coleoptiles of *Avena* (method of WENT (4), improved by VAN DER WEY (6)).

Table 4 and fig. 2 show the results of the transportation-experiments in the light and in the dark. *The quantities of growth-substance transported through hypocotyls in the light, certainly are not less than those transported in the dark.*

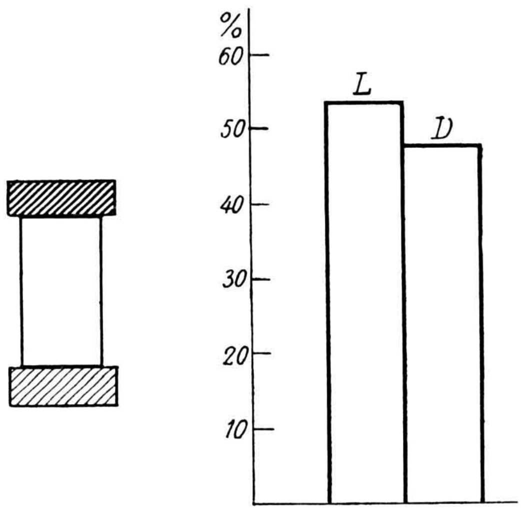

Fig. 2. Relative quantities of growth-substance transported through cylinders of hypocotyls of *Raphanus*, in all-round light and in the dark. On the left a scheme of these experiments: a cylinder of a hypocotyl with on its top a block of agar containing growth-substance and on its base a receiving block of agar. Column *L*: in all-round light. Column *D*: in the dark.

I varied this experiment in various ways (length of the cylinders, concentration of the growth-substance, transportation time), but always with the same result.

TABLE 4.

Quantities of growth-substance transported through cylinders of hypocotyls of *Raphanus*. In all-sided light and in the dark. Sum of 2 × 21 experiments (504 cylinders). Length of the cylinders in various experiments: 2, 6 and 8 mm. Concentration of the growth-substance: 30°, 100° and 300°. Transportation time: 1, 2 and 3 hours.

All-sided light	Dark
288° (**53 %**)	254° (**47 %**)

Ad b. As the quantity of growth-substance transported in the light is not less than in the dark, the experiments of fig. 1 therefore have proved that *in the dark the cells are more sensitive to growth-substance than in the light.* In hypocotyls exposed from one side, it is therefore very probable that the shaded side is more sensitive to growth-substance than the illuminated side. Even when the growth-substance is equally distributed and the shaded side has a greater sensitivity to growth-substance, the hypocotyl will still grow towards the light. It seems to me that there is a resemblance between the principle of BLAAUW's theory of the light-growth-reaction and the phenomenon of the greater sensitivity of the cells to growth-substance in the dark than in the light.

At this place an experiment with the *plant's own growth-substance* has to be mentioned. From seedlings of *Raphanus*, exposed to the light on all sides by revolving on the vertical axis of a clinostat, one of the two cotyledons is cut off. One series of these plants is placed in the dark, an other series remains on the clinostat in the light. Seven hours after that, the plants in the light had a mean curvature (positive traumatotropic) of 4°.3, whereas the plants in the dark had a mean curvature of 13°.4. The plants which had been in the light were now put into the dark. Two hours after that their curvature had increased from 4°.3 to 17°. It will be clear that traumatotropic curvatures like these develop best when light-grown plants are put in the dark.

II. *The distribution of the growth-substance.*

i. *The distribution after one-sided exposure of the hypocotyl.*

It was F. W. WENT who showed first that much more growth-substance may be obtained from the shaded side of one-sidedly exposed tips of *Avena*, than from the illuminated side. He exposed the plants on one side, cut off the tips and placed them each on two blocks of pure agar, so that the growth-substance of the "light" and the "shade" side could be gathered separately.

I changed this method by placing the tips or hypocotyl-cylinders of unexposed plants on razor blades, in such a manner that the basal cut of the tip or cylinder was divided exactly into two equal parts. On each part I hung a block of agar. On each blade twelve tips or cylinders were placed. The whole is put under a little glass jar, covered inside with wet filter-paper. One side of the cover remained unscreened to let in the light; this side was placed against the glass door of the thermostat.

In order to see whether this method gives the same results as that of F. W. WENT, I put tips of *Avena* with a length of 6 mm. on the blades, hung the little blocks of agar on both the sides and exposed one side with 750 M.C.S. (a quantity of light of 50 candles at a distance of 1 meter during 15 seconds). Three hours later the blocks were taken from the tips and analysed. *The results shown in table 5, agree with the results of* WENT.

TABLE 5.

Quantities of growth-substance extracted from the light and the shade side of tips of *Avena*, exposed with 750 M.C.S. Mean of 12 tips. Extraction time: 3 hours.

Light side	Shade side
4.5 ± 1.2	10.2 ± 2
6.8 ± 1.5	9.3 ± 1.9

I performed the same experiment with tips of *Raphanus*. From hothouse plants the cotyledons were cut off and tips of 1 cm. were put on the blades. Blocks of agar were put below both sides. While the tips were standing on the blades (3 hours), they were illuminated on one side by a lamp of 500 Watt at 75 cm. distance, during one or two hours. Between the lamp and the object a water screen, with a depth of 20 cm., was placed. The tips on the blades were put under little glass covers. These glass covers stood in a thermostat, with a temperature as high as that of the darkroom (22° C.). The thermostat has a double door of glass. When the light has passed the water basin, it has to pass the double glass door before it enters the thermostat. Heating of the experimental objects by the lamp was, therefore, not to be feared. Table 6 gives the results of these experiments. As with tips of Avena, *the tips of Raphanus contain less growth-substance on the "light" side than on the "shade" side. In the dark the quantity of growth-substance on both sides is equal.*

TABLE 6.

Quantities of growth-substance extracted from both sides of tips of *Raphanus* in one-sided light and in the dark. Extraction time: 3 hours. Mean of 12 tips.

One sided light			Dark	
Light side	Shade side	Time of exposure	One side	Other side
1 ± 1	4.3 ± 1.5	2 hours	5.1 ± 0.8	5 ± 1.5
0.8 ± 1	5.8 ± 1	1 hour		

Now it was necessary to investigate whether or not the presence of the tip is necessary for the mentioned distribution of growth-substance in hypocotyls, which were illuminated from one side. Therefore the distribution of growth-substance was tested in hypocotyl-cylinders exposed from one side. In all these experiments the cylinders had a length of exactly 6 mm. and were cut to this length with the apparatus described by VAN DER WEY (7). The cylinders of the hypocotyls were cut 3 mm. (and sometimes also 9 mm.) under the place of insertion of the stalks of the cotyledons. The hypocotyls were of plants grown in the hothouse and were put into the darkroom one night before the beginning of the experiments. About 4 hours before beginning the experiments the plants were decapitated or the cotyledons were cut off.

These cylinders were placed on the razor blades in the usual way. Two separate blocks of pure agar were placed under each cylinder and the top of each cylinder was covered with a block of agar containing auxin (scheme in fig. 3). After that, one series of the prepared cylinders was exposed on one side by the same lamp as the tips of *Raphanus*, during the whole extraction-time of 3 hours. From table 7 and fig. 3 it is

TABLE 7.

Quantities of growth-substance extracted from both sides of cylinders of hypocotyls of *Raphanus* in one-sided light and in the dark. Sum of 8 experiments in the light and 2 in the dark. Sets of 12 cylinders. Extraction time: 3 hours. Concentration of the auxin: 250°. Auxin distributed equally over the surface of the top.

One-sided light			Dark		
Light side	Shade side	Number of experiments	One side	Other side	Number of experiments
53 (**37** %)	88 (**63** %)	8	20 (**50** %)	19.8 (**50** %)	2

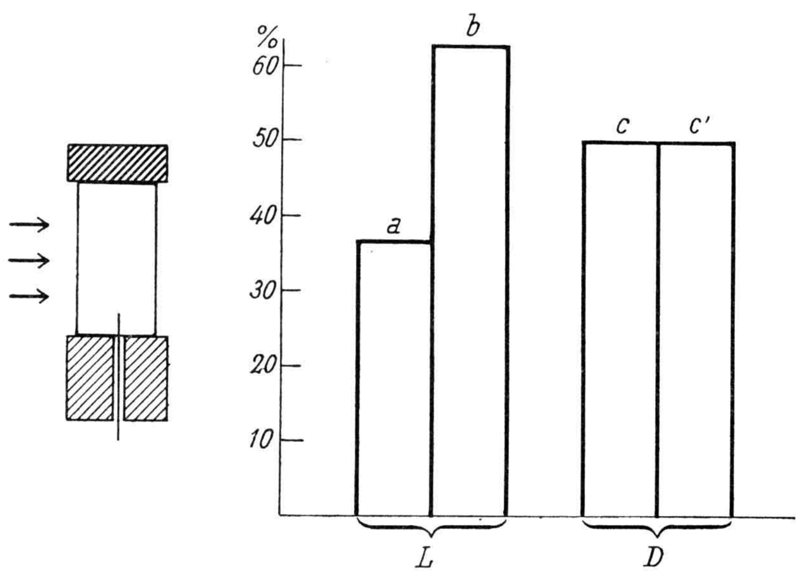

Fig. 3. Relative quantities of growth-substance transported to 2 separated blocks of agar on both sides of one-sidedly exposed cylinders of hypocotyls of *Raphanus* and the same experiment in the dark. The 2 columns *L*: in one-sided light; *a*: light side; *b*: shade side. The 2 columns *D*; in the dark; *c*: one side; *c'*: the other side. Auxin applied all-sidedly. On the left a scheme of the experiments: a cylinder of a hypocotyl with on its top a block of agar containing auxin and on its base 2 separated receiving blocks of agar. Arrows: direction of the light.

apparent that, *in agreement with the experiments with tips, the greatest quantity of the growth-substance is extracted from the shaded side.* In the dark the blocks on both sides of the cylinders contain equal quantities of growth-substance. *We see therefore that the presence of the tip is not necessary for the unequal distribution of the growth-substance in hypocotyls of* RAPHANUS.

ii. *The way in which this distribution is brought about.*

A larger quantity of growth-substance in the shaded side may occur:

a. When the stream of growth-substance has a direction parallel to the longitudinal-axis of the hypocotyl, but with a relative inhibition of this stream of growth-substance on the light side.

On fig. 2, however, one can see, that the transport of growth-substance in cylinders of the hypocotyls of *Raphanus* is not less intense in the light than in the dark.

b. When the stream of growth-substance has a direction not parallel with the longitudinal-axis of the hypocotyl, but a transverse direction to the shaded side.

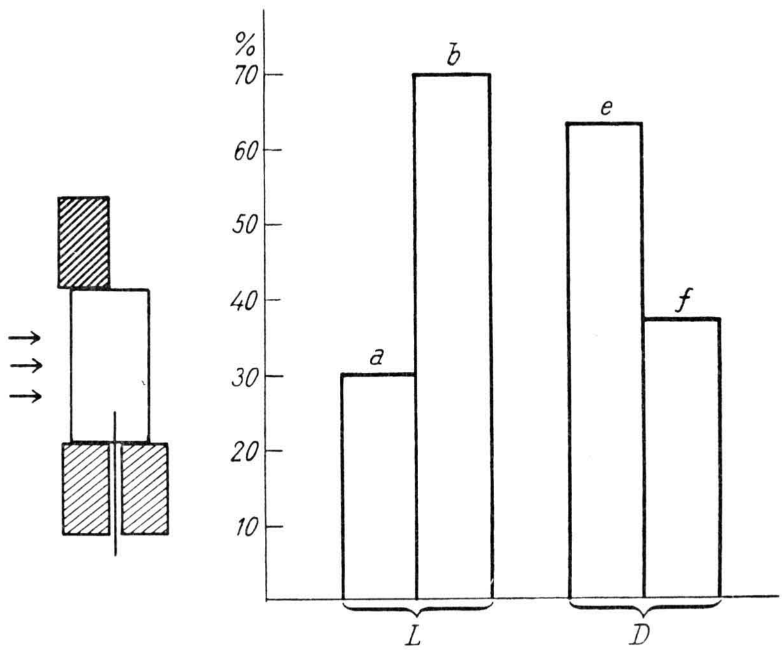

Fig. 4. Relative quantities of growth-substance transported to 2 separated blocks of agar on both sides of one-sidedly exposed cylinders of hypocotyls of *Raphanus*; and the same experiment in the dark. The 2 columns *L*: in one-sided light. *a*: light side; *b*: shade side. The 2 columns *D*: in the dark. *e*: top block side; *f*: the other side. Auxin applied one-sidedly. On the left a scheme of these experiments: a cylinder of a hypocotyl with on its top a block of agar containing auxin, put on one side, and on its base 2 separated receiving blocks of agar. Arrows: direction of the light.

TABLE 8.

Quantities of growth-substance extracted from both sides of cylinders of hypocotyls of *Raphanus*. Top covered on one side with blocks of agar containing auxin. The side with the top block is exposed to a lamp of 500 Watt distant 75 cm, during 3 hours transportation time. Controls in the dark. Length of the cylinders 6 mm. Concentration of auxin 250°. Mean of 12 cylinders.

No.	One-sided light		Dark	
	Light side (top block side)	Shade side	Front side (top block side)	Back side
21115	3 ± 1.9	10 ± 2.3	11 ± 3	7.6 ± 1.8
21114	3.3 ± 2	10 ± 2	12.5 ± 2	6.6 ± 1.6
— 1)	2 ± 0.8	9.5 ± 2	—	—
21101	3.6 ± 1	6.6 ± 1.8	7.7 ± 1.1	6.1 ± 1.1
21031	5.1 ± 2	9	9.3 ± 3	4.5 ± 2
— 1)	2.7 ± 1.1	7.1 ± 1.5	5 ± 2.5	4.5 ± 2
21015	7.7 ± 1.3	11.1 ± 2.7	10.2 ± 3	3.9 ± 1.7
— 1)	1.5 ± 1.2	5.0 ± 3	5.4 ± 1.2	1.0 ± 0.9
21014	2.8 ± 0.6	7.3 ± 1.3	5.6 ± 1.1	5.6 ± 1.6
Sum	31.7 (30 %)	75.8 (70 %)	67 (63 %)	40 (37 %)

If this is the case, the following experiment will show it. Cylinders of hypocotyls with two blocks of pure agar below are prepared as in the experiment of fig. 3. On top of each cylinder is placed a small block of agar containing growth-substance, covering only a trifle less than half the top. This top block is placed exactly above one of the blocks below. We can now illuminate one series of the so prepared cylinders on the side of the top block (scheme in fig. 4). An other series is kept in the dark. In the experiment in the dark we may now expect to find most of the growth-substance in the block just below the top block. In the experiment in the light (exposure on the side of the top block) on the contrary, we may expect to find most growth-substance in the other block, because this block is on the dark side. Table 8 gives the whole of the results of these experiments. Fig. 4 once more gives the relative values of the sums of the experiments. We see immediately that the results of these experiments agree entirely with the expectations.

These experiments directly show that the growth-substance in the one-sidedly exposed hypocotyl is drawn to the shaded side. The idea of F. W. WENT (4, p. 107), "Einseitig einfallendes Licht lenkt den sonst

1) Cylinders cut 6 mm more basal than usual.

allseitig aus der Spitze kommenden Wuchsstoffstrom derweise ab, dass die Lichtflanke sehr wenig und die Schattenflanke einen Überschuss des Wuchsstoffes empfängt" has therefore been shown to be right, also for *Raphanus*.

One will find a summary of the principal results obtained in the figures of this publication. The phototropic curvature is caused by two reactions:

1. The greatest part of the growth-substance is drawn to the shaded side (WENT's theory).
2. Added to this, the sensitivity of the cells to growth-substance is greater on the shade side than on the light side.

A more detailed report will be published later on.

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Botanical Laboratory.

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Medicine. — *On anophelism without malaria around Amsterdam.* IV. *The pattern of the dorsal surface of the ova in the two races of A. maculipennis.* By A. DE BUCK and N. H. SWELLENGREBEL. (From the Zoological Laboratory, Department of Tropical Hygiene, Royal Colonial Institute, Amsterdam.) (Communicated by Prof. W. A. SCHÜFFNER.)

(Communicated at the meeting of December 17, 1932).

HACKETT, MARTINI and MISSIROLI (1932) recently described two races of *Anopheles maculipennis* in Italy and some parts of Germany differing by the colour pattern of the dorsal surface of the eggs. One race, associated with malaria, has what they call "dappled gray eggs", the other, no malarial vector, is characterised by its "barred eggs".

The *"dappled gray eggs"*, they describe as showing "the blue-gray finely tesselated dorsal surface ... dappled rather uniformly with rounded