

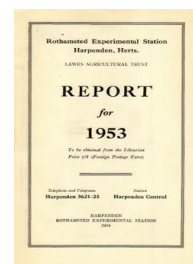
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## Special Reviews

### Rothamsted Research

Rothamsted Research (1954) *Special Reviews* ; Report For 1953, pp 167 - 187 - DOI: <https://doi.org/10.23637/ERADOC-1-75>

## BEEES AS POLLINATORS OF FRUIT AND AND SEED CROPS

By

C. G. BUTLER AND J. SIMPSON

### I. INTRODUCTION

It has been shown that a number of important agricultural crops require the services of insects as pollinators. Whilst this does not apply to the major crops required for human consumption, such as cereals and potatoes, it is particularly important in the case of the legumes, which play such an essential part in agricultural economy. Many other crops require insect pollination in order to produce fruit or seed, of which such horticultural crops as brassicae and orchard fruits are good examples.

It should also be mentioned that many grasses and forest trees, which rely on wind pollination, are frequently visited by large numbers of pollen-gathering bees (Synge, 1947).

Although adequate data are not yet available, it is probable that in many parts of Britain today, as a result of intensive cultivation, the number of wild pollinating insects is insufficient to ensure full production. However, the distribution of honeybee colonies is by no means ideal for this purpose, since the great majority are kept in the immediate neighbourhood of large cities and not in those areas where insect-pollinated crops are extensively grown. This state of affairs is harmful both to the farmer and to the beekeeper, whose honey yield suffers as a result of excessive competition for limited supplies of bee forage. Its rectification is, however, clearly a matter of organization rather than of research, and the research worker is more concerned with those cases where, despite the presence of bees, pollination still remains inadequate.

### II. INSUFFICIENT POLLINATION

Many insects, including bees, visit the nectaries of flowering plants in search of food. It has been shown by Wykes (1952*c*) that honeybees prefer solutions containing sucrose, glucose and fructose to solutions of the same total concentration of any single one of these sugars. The fact that most nectars contain these three sugars in major proportions (Wykes, 1952*a*; 1953*a*) may, therefore, indicate an aspect of the mutual adaptation between plant and bee. Wykes (1953*b*) has also found that the removal of nectar from the nectaries of some plants stimulates further secretion—an interesting example of economy on the part of the plant.

In most cases the nectaries are situated within the flowers themselves and, when approaching them, the insects usually effect the pollination of the flowers concerned. Floral nectaries are probably more attractive than extra-floral ones, and also the more readily found on account of the colours and scents of the flowers. It has been shown by Oettingen-Spielberg (1949) that worker honeybees searching for new sources of food are particularly attracted to small, coloured objects. This has been confirmed by Butler (1951), who

has also shown that bees will alight on such objects much more readily if suitable scents are also present. Furthermore, he has shown that bees that have been visiting a crop of scented flowers for some time will hesitate to enter them if the perfumes of the individual flowers are experimentally masked with another perfume, even with one which is normally attractive to bees. This probably explains the observation of Butler, Finney and Schiele (1943) that many bees are deterred, at least temporarily, from continuing to visit flowers when they are sprayed with insecticidal and fungicidal mixtures containing scented materials which are not, in themselves, strongly repellent to bees.

Bees are especially important as pollinating insects because, both as larvae and as adults, they are entirely dependent upon nectar and pollen for their food, and numerous visits have to be made to flowers to collect them. The branched hairs on their bodies, which enable them to collect pollen, also increase their pollinating efficiency. The honeybee is especially valuable because its colonies contain thousands of individuals which can readily be moved to those places where they are required.

Failure of bees to work on crops is often explicable by lack of nectar or by the presence of nectar which is too dilute to attract them. Some plants consistently produce nectar which, both in quantity and quality, is attractive to bees, whilst others cannot be relied upon to do so. Such variations in nectar secretion can usually be attributed to weather conditions, even to those of the previous year, through their effect on carbohydrate accumulation (Wykes, 1952*b*), but may also be affected by the availability of soil nutrients (Beutler, 1953). Ryle (1954*a*, 1954*b*) has investigated the effect of fertilizer treatment on nectar secretion in mustard, buckwheat, apple and red clover. She showed that with apple-trees the mean quantity of sugar produced per flower was significantly increased by extra potash. In sand-culture experiments with red clover, mustard and buckwheat, in which the levels of nitrate, phosphate and potash were varied, any treatment which checked growth at flowering, apart from a shortage of potash, increased the yield of nectar. However, with the clonal material used in the red-clover experiments, it was found that the differences caused by the fertilizers were small in comparison with those between clones. This suggests that it may be possible to select strains of red clover which, whilst retaining their present good vegetative qualities, will also have improved nectar-secreting properties.

It is also possible for flowers to contain nectar which is not available to all pollinating insects. Thus inadequate pollination of red clover by honeybees can be due to the long corolla-tubes of the flowers of this plant, which make it difficult, if not impossible, for the bees to reach the nectar unless it is very plentiful. The longer-tongued species of bumblebees, such as *Bombus agrorum* and *B. ruderatus*, are better able to pollinate this plant, but the short-tongued species, such as *B. terrestris* and *B. lucorum*, are often actually harmful, since, by biting holes at the bases of the corolla-tubes, they obtain the nectar without making contact with the stamens and stigma, and enable honeybees to do likewise. These facts have recently been verified by Free (1952). Ribbands (1951) has shown that in order to obtain maximum pollinating efficiency

colonies of honeybees should be placed as close as possible to the crop, since the amount of foraging in bad weather is considerably reduced when the bees have to fly even short distances.

Numerous cases have been recorded of crops which would otherwise be reasonably attractive to bees being neglected in favour of still more attractive crops, which have sometimes actually been weeds. For example, Vansell (1942) has described a case of competition, a multiple case, between the flowers of apple, peach, nectarine, plum, sour cherry, winter Nelis pear and Bartlett pear, in which the two varieties of pear were almost completely neglected by the bees present in favour of the apple and other flowers. Butler (1945*a*) has described similar cases of competition between pear and hawthorn, in which the pear blossom was neglected in favour of the hawthorn, and also between greengage and dandelion, in which the dandelions received the bulk of the bee visits. Hammer (1949) showed that red clover, even when it was yielding nectar well, was liable to be deserted in favour of mustard, lucerne or carrot. He found that this difficulty could be overcome by providing more bees than the competing crops could carry.

Bees foraging for nectar may in some instances be ineffective as pollinators if the floral structure permits them to reach the nectaries without touching the stamens and stigma. Thus some varieties of apples have long, erect stamens beneath which bees can crawl to reach the nectaries. In the case of flax many bees learn to approach the nectaries by thrusting their tongues between the petals from the back of the flower (Gubin, 1945), in which behaviour they are possibly encouraged by the fact that flax petals are extremely loosely attached and perhaps do not provide an adequate support for a bee (Simpson, 1949). In the same sort of way nectar-gathering honeybees rarely accomplish the tripping of lucerne flowers, which is necessary for their pollination, having learned to obtain the nectar without thrusting their heads into the corolla-tubes (Tysdal, 1940). Honeybees often take a little time to learn such irregular methods of obtaining nectar (Butler, 1949), and Dadant (1951) has suggested changing the colonies on the crop regularly to reduce the effects of such learning.

Extra-floral nectaries on plants also allow insects to obtain nectar without effecting pollination. It is surprising, therefore, that in some plants, such as the field bean and cotton, such nectaries are active at the time of flowering.

Where it is sufficiently abundant, pollen of itself may attract pollinating insects. This occurs with a few nectarless plants such as poppies. Since pollen-collecting bees almost invariably pollinate the flowers which they visit, most pollinating difficulties could be overcome by increasing the number of bees gathering pollen from the crop. This can be done by increasing the total bee population in the district. About one colony of bees per acre is usually sufficient to ensure the pollination of crops where nectar-gatherers are the effective agents (Hutson, 1926), but advantages have been shown in increasing this number to three to four per acre in the case of red clover, from which the bees often obtain insufficient nectar (Hammer, 1950), and to five per acre with lucerne, where nectar-gatherers do not pollinate the flowers (Dadant, 1951).

Many more individuals are usually necessary to gather the

nectar required by a colony of honeybees than are required to collect its pollen. It follows, therefore, that if the population of honeybees in any given area is increased, until the number of pollen-gathering bees is sufficient to pollinate a given crop, the colonies used are unlikely to give a satisfactory return of honey and may even require to be fed. The economics of this system of ensuring pollination in any particular instance should, therefore, be carefully examined. The females of many solitary bees, for example *Megachile* sp., are mainly concerned with pollen collection when foraging, and are, therefore, probably more useful as pollinators of crops such as lucerne (Franklin, 1951), from which honeybees can obtain nectar without effecting pollination. Attempts have already been made in America to propagate *Nomia melanderi* for this purpose (Menke, 1952); otherwise this possible method of solving the problem appears to have received little attention.

The possibility of varying the proportion of pollen to nectar loads collected by honeybee colonies has been considered. There is some evidence that this can be done by creating a pollen shortage in the hive by using a pollen trap to remove pollen from the legs of returning foragers (Hirschfelder, 1951; Lindauer, 1952). Most traps, however, remove only about 20 per cent of the loads of pollen brought in by bees, and although a trap which removes as much as 75 per cent has been produced at Rothamsted, the obstruction which it causes reduces the foraging level of the colony excessively. Unless this difficulty can be overcome, it seems improbable that pollen trapping will prove to be useful in this respect. Pollen collection may also be increased by adding to the amount of brood in the colony, but this, too, involves considerable beekeeping difficulties.

### III. CROSS-POLLINATION

Many plants of considerable economic importance are wholly or partially self-sterile, or possess mechanisms which hinder self-pollination. It is important, therefore, that pollinating insects should carry pollen from plant to plant.

Individual honeybees do not forage over the whole of the area within flight range of their hive, but tend to return continually to a small part of this area (Müller, 1882). This type of behaviour is also shown by other insects (Minderhoud, 1951), and may well be a characteristic of foraging animals in general. Individual bees also frequently restrict their activities, at least for a time, to the flowers of one of several available species of plants (Aristotle).

It is obviously desirable that the foraging areas of individual honeybees should be large where the transference of pollen between trees, often between widely separated trees (as in orchards interplanted with compatible varieties), is necessary; and that they should be small where transfer of pollen between adjacent plots, as when growing seed of compatible varieties of brassicae, must be avoided.

Butler (1943) described honeybees restricting their foraging on a crop to areas of 5 yards or less in diameter, and the existence of foraging areas of similar size was deduced by Crane and Mather (1943) from a study of the distances necessary for isolation between

crops of different varieties of radish. It was pointed out by Butler (1943) that bees foraging in such small areas cannot be responsible for cross-pollination in orchards, and, since the necessary transfer of pollen between trees does occur, he postulated (1954*b*) the existence of an additional "wandering" population of bees. He considered that these were probably mainly young bees which had not yet found satisfactory foraging areas. It was known, however, that bees tend to extend their foraging areas and to wander when the crop on which they have been foraging begins to fail. Thus in an experimental field, which extended over a considerable area, in which artificial flowers (dishes of syrup) were spaced 20 yards apart from one another, Butler, Jeffree and Kalmus (1943) found that honeybees which were accustomed to collect food from particular dishes moved elsewhere when the supply of syrup in these dishes failed, but, nevertheless, returned from time to time to these dishes and examined them. If the supply of syrup was subsequently replenished and maintained, the bees would often be found to have enlarged their original foraging areas to include several dishes, some of which they visited only occasionally. Similarly, Ribbands (1949) found that honeybees that were gathering pollen from Shirley Poppies spread their activities over a greater number of flower-heads as the supply of pollen became exhausted. From this and other observations with different crops he came to the conclusion that the size of a honeybee's foraging area is liable to continuous change, and is dependent at any given moment on the extent to which she is satisfied with the return for her foraging activity. Since von Frisch (1934) has shown that such satisfaction is related to the previous foraging experience of a bee, it is probable that as different bees have had different experiences they are liable to be variously satisfied, and Ribbands (1949) has concluded that one is likely to find a wide range of sizes of foraging areas amongst any population of honeybees working on any crop at any given time. Thus both Butler and Ribbands agree that the sizes of the foraging areas of individual bees vary from time to time, but explain this phenomenon in different ways.

Butler (1945) supposed that the proportion of "wandering" bees could be raised by increasing the density of bees on the crop, but Ribbands (1953) has concluded that the effect of competition on the sizes of foraging areas is unpredictable from the available evidence. This problem, which clearly has an important bearing on orchard pollination, still remains to be solved experimentally.

#### IV. THE DIRECTING OF HONEYBEES TO CROPS

Von Frisch's (1925) discovery that successful foragers are able to communicate the scent of the flowers from which they have been gathering food to other bees has led to attempts to direct honeybees to crops which need to be pollinated. The method used has been to feed syrup, containing the scents of the flowers of the crops requiring to be pollinated, to colonies of bees.

Foragers that are seeking food are attracted to flowers by their movement (Wolf, 1937) and by their colour and scent (Butler, 1951). Ribbands (1949) has demonstrated that when a honeybee knows of more than one source of food she appears to select the best

of these at any given time, and von Frisch (1946) has shown that she is able to communicate to other members of her colony the positions of any of these sources.

Close observations suggest that colonies of honeybees possess very effective methods of finding and exploiting the best of the crops within their foraging range, so that although it might be possible to mislead them into pollinating one of the poorer crops, no increase in honey yield could result from this procedure. However a colony's methods of finding the best crops available do not, in fact, appear to be as effective as one might expect them to be, as it has been noted that colonies in the same apiary will frequently collect the bulk of their food from very different sources (Synge, 1947). It has also been shown that colonies of bees that have been moved to a heather area before the heather flowers have opened, and have commenced to forage on other kinds of flowers, have failed to transfer their activities to the much more abundant heather flowers when these became available (Moore-Ede, 1947). It seems possible, therefore, that when colonies of bees are directed to crops to pollinate them their honey yields may occasionally be increased.

In early practical experiments to direct bees to crops Russian workers, such as Veprikov (1936), claim to have obtained considerable increases in the number of bees visiting the experimental crops and in the amount of seed produced. However, later investigations by von Frisch (1947) produced less definite results; in his experiments the number of honeybees foraging on the experimental crops appear almost invariably to have been increased, but the figures obtained for set of seed, and for seed yield at harvest, are less satisfactory. On crops other than red clover increased honey yields (allowance being made for the sugar fed) were obtained. In the case of red clover, however, the effects on honey yield were not significant. This suggests that although von Frisch was successful in directing the bees to red clover, they were unable to obtain any more nectar from these flowers than they would have done from others.

Von Frisch (1947) pointed out that directing bees to crops from which they cannot obtain nectar is not likely to result in much additional pollination of the crop. He, therefore, suggested that in such cases it might be possible, and more profitable, to attempt to direct pollen-gathering bees to the crop rather than nectar-gatherers, by feeding syrup scented with the pollen of its flowers. Unfortunately, experiments at Rothamsted to direct bees to red-clover crops, by feeding red-clover pollen in syrup, have produced no evidence that the proportion of pollen gathered from red-clover flowers can be increased by such treatment.

Von Frisch (1947) also showed that it is more effective to feed scented syrup to bees outside the hive than inside. Some unpublished observations by Butler suggest that this may be due to the very much greater tendency of bees that have collected food in the light to perform recruiting dances, and also that intermittent feeding is likely to be more effective than continuous feeding, as most of the dances are performed by the first few bees which visit the feeder.

## V. USE OF HONEYBEES AS POLLINATORS IN PARTICULAR INSTANCES

### (a) *Orchard pollination*

Brittain *et al.* (1933) have pointed out that the number of colonies of honeybees required to yield the necessary proportion of bees to flowers in an orchard depends on many factors, including, of course, the area of the orchard and also the amount of bee forage, other than that provided by the fruit-trees, available in and around the orchard. Butler (1948) recommended that a group of colonies should be placed in the centre of each 15–20 acres or orchard. Although the validity of the suggestion that this method of placing the colonies increases the degree of competition between the bees and also increases their tendency to wander from tree to tree, and thus to effect cross-pollination, has not yet been adequately demonstrated, there is no doubt that it possesses certain advantages. By keeping the colonies away from the edges of the orchards it probably reduces the tendency of the bees to forage outside them, and further it enables the grower to determine, over a period of time, the number of colonies of a given strength necessary to produce an adequate set of fruit, since if an insufficient force of bees is present the set of fruit falls off at some distance from the hives, and when an even set of fruit has been obtained throughout an orchard the force of bees is probably correct (Butler, 1942). Grouping of the colonies together is also advantageous to management both by the beekeeper and the grower. In spite of Ribbands' (1951) observations of the large diminution of foraging in bad weather with increased flying distance, it is unlikely that the method of locating colonies suggested by Butler (1948) will result in any serious diminution of foraging activity, since the radius of a circle of an area of 20 acres, in the centre of which it has been suggested that the colonies should be placed, is only 176 yards. Larger groupings, however, are undesirable.

### (b) *Pollination in confined spaces*

Colonies of honeybees are sometimes used to cross-pollinate such crops as peaches in glass-houses (Thompson, 1940). Unfortunately, however, although honeybees have been found to be very satisfactory for such purposes, and to save much manual labour, the condition of the colonies used tends to deteriorate very rapidly and the foraging force to diminish during the first few days of confinement to the house, on account of many of the bees dying in attempts to escape. However, the young bees which replace the original foragers show a much reduced tendency to behave in this way.

Recently colourless nylon screen-cages have been found useful in work on the pollination of red clover and other crops, as well as for work on the breeding of brassica varieties. It has been found at Rothamsted that bees behave well in these cages and that normal plant growth is maintained within them. Indeed it seems probable that this type of cage may prove extremely valuable in plant breeding.



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## PHYSICAL PROPERTIES AND CONTACT TOXICITY OF DDT AND SOME RELATED COMPOUNDS

By

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Research on biologically active compounds, including insecticides, is often done by making a group of similar chemicals and testing them by some standard method. This may lead to the discovery of new insecticides. But the aim is sometimes to correlate chemical structure with toxicity in the hope of finding some general rule by which the toxicity of any chemical in the group may be foretold.

The physical as well as the chemical properties of a compound can affect its toxicity, and when chemical structure is changed, physical properties are nearly always changed as well; so that it may sometimes be misleading to relate toxicity directly to chemical structure unless changes in the important physical properties are small.

Some of the work done in the past few years at Rothamsted has been aimed at finding out what effect the physical form can have on toxicity, and what physical properties are desirable in an insecticide when it is applied directly to the insects' bodies. This work is academic, but may in the end have some effect on the way insecticides are made up for field use.

All the compounds we used are chemically related to DDT. They are all crystalline solids which do not dissolve in water. They are all contact poisons. This means that the insects can be killed without having to eat the poisons; contact with the insects' bodies is enough. None of the poisons give off vapours that can kill the insects.

Two or more types of aqueous suspensions were made with each compound. One type (colloid) contained very small particles of supercooled liquid poison, probably about 0.0001 mm. in size. The others contained crystals, often about 0.05 mm. These crystals were uniform, but the size varied from one compound to another; in some cases several different types of suspension were made of a single compound, each containing uniform crystals of characteristic size.

The toxicity of each suspension was found by a method which involves dipping saw-toothed grain beetles (*Oryzaephilus surinamensis*) for a few moments in the suspension (McIntosh, 1947a). After this the suspension is drained off, and the beetles are left with a coating of poison sticking to them. The dipping does not drown the insects; they are kept for 24 hours or more after dipping, and then counts are made to see how many have died from the poison. It is important that the temperature of the insects is kept constant during this time, because changes in temperature nearly always affect the kill.

In this way the suspensions were compared in pairs; a suspension of crystals of each poison was compared with the same poison in

colloidal form. This review discusses how the difference in toxicity between colloid and crystals may be related to the physical properties of the poison.

DDT was one of the compounds with which several different suspensions of crystals were made, each containing crystals of a different size. Crystals of DDT are needle-shaped or plate-shaped; the crystal size was varied from about 0.06 mm. to about 0.4 mm. When these suspensions were compared on grain beetles kept warm (27° C.) after dipping, the colloid was always the least toxic suspension; the longer the needles, the more toxic they seemed to be (McIntosh, 1946). The longest needles were about fifteen times more toxic than the colloid. This was unexpected, but the immediate cause was not hard to find. When the insects are taken from the suspension, poison sticks to them; it can be washed off, and the amount retained can be found by chemical analysis. This showed that the insects retain much more poison from a suspension of long needle-shaped crystals than from a suspension of colloidal particles. The extra dose received was in fact almost enough to account for the higher toxicity of the suspension of crystals (McIntosh, 1947*b*). Differences in toxicity amongst the other suspensions of DDT crystals can be explained in the same way; crystal size decides retention. Tests with other compounds besides DDT suggest that retention of this sort is purely mechanical. Retention by one insect species depends on crystal size only; different poisons with crystals of the same size are retained equally well. Plate-shaped crystals are not retained so well as needle-shaped crystals. Poorest retention was found with plate-shaped crystals of about 0.025 mm., and not with the very smallest particles. With some poisons there is no method for micro-analysis. In such a case the retention can be guessed by comparison with some other compound that gives analyzable crystals of the same size.

The results of all comparisons of toxicity by dipping must be corrected one way or the other to allow for differences in retention.

It may seem at first sight as if the results of the tests with DDT can be completely explained by differences in retention. This is not so. A very short description of what insect cuticle (skin) is like may make this clearer. Cuticles vary in structure from species to species, and even from one area of a single insect to another. But there is always a thin waxy layer on the very outside (Wigglesworth, 1948; Beament, 1948). The first thing a contact poison lying on the cuticle must do to get into the insect is to dissolve in this wax layer. Without this, nothing can happen to the insect. For this reason the need for a contact insecticide to be soluble in fat has often been stressed.

With DDT it is thought that there are certain spots on the cuticle surface which are more easily penetrated than the rest, or which form short-cuts to the site of action of DDT inside the insect (see, for instance, Schaerffenberg, 1949; Wiesmann, 1949; Fisher, 1952). Poison applied to them kills the insect more efficiently than the same amount of poison applied anywhere else. The wax covers the sensitive spots as well as the rest of the cuticle, and so the first step in penetration must be the same everywhere, whether the insect dies as a result or not.

The wax layer is very thin, and must soon become locally

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saturated beneath and round about particles of poison that are in contact with it. If saturation can be kept up long enough, especially on a sensitive spot, the insect will die.

After insects have been dipped, the film of poison sticking to them becomes dry. With colloidal poison the film is not even, but takes the form, to begin with anyway, of little blobs of supercooled liquid poison. The chances of hitting a sensitive spot with a blob or with a crystal must be about the same. One might naturally expect that the poison from the blobs would dissolve more quickly in the wax than the poison from the crystals; colloidal poison should be more toxic than crystals, or should act more quickly. However, the two forms of DDT are in fact almost equally toxic.

Counts of kill are usually made one or two days after treatment. The choice is largely one of convenience. But it did not seem to matter whether they were made after  $1\frac{1}{2}$  hours or 72 hours; the ratio of toxicities was always the same (McIntosh, 1949). So we have the unexpected fact that the speeds of solution of the two forms of DDT in wax are, as far as can be judged from the biological tests, nearly the same. Speed of solution does not seem to decide speed of kill.

What has been said so far applies to insects that are kept warm ( $27^{\circ}$  C.) between dipping and counting. If the insects are treated with the same two forms of DDT and then kept cool ( $11^{\circ}$  C.) instead of warm, the relative toxicity is reversed; the colloid is now more toxic than the crystals by about the same amount as it is less toxic to the warm insects. Tests by injection of suspensions into larger insects give similar results, and suggest that the difference in toxicity at  $11^{\circ}$  C. is largely a difference in speed of action; if the injected insects are kept cool for long enough, the kill from the crystals catches up on the kill from the colloid (McIntosh, 1951a). The process of dissolving is slowed down in cool insects, but it is slowed down more for the crystals than for the colloid. The physical theory of very small particles supports the idea that they should be relatively more toxic at lower temperatures (McIntosh, 1951b).

One effect of cooling the insects is to accentuate the difference in speeds of action between small and large particles, making it easier to measure. Other compounds related to DDT behave in somewhat the same way when tested as contact poisons on *O. surinamensis* kept cool after treatment. The colloidal form is always more toxic than crystals, but the size of the difference in toxicity varies from one analogue to another.

Two properties of dissolving materials might be expected to affect this difference in toxicity.

Firstly, the deposits left by the colloidal poisons are made up of globules to begin with, but often crystallize later. The speed at which this happens varies from compound to compound, and can be measured in *in vitro* tests. If the deposit crystallizes quickly, it is soon not very different from the deposit left by a suspension of crystals; the difference in toxicity between colloid and crystals is likely to be small.

Secondly, if it is in fact necessary for poison to saturate the wax layer, then the speed at which a poison can dissolve in the wax may be more important than the solubility itself. It is possible to measure

the time it takes for crystals of a poison to bring about saturation of olive oil *in vitro*. This figure was taken as a guide to their speed of solution in insect wax. It was not possible to measure the speed of solution of deposits from colloidal poisons in olive oil; they dissolve quickly, and it was assumed that they all dissolve at the same speed. Different poisons with crystals of the same size do not necessarily bring about saturation at the same speed. If the crystals dissolve slowly, the difference in toxicity between colloid and crystals is likely to be large.

When allowance is made for differences in retention, the analogues fall into two groups. Each compound in the first group shows a difference in toxicity of about eight times; the colloid is about eight times more toxic than the crystals if counts are made one day after treatment. In the second group the differences in toxicity between colloid and crystals are very much bigger; the values found lie between thirty and eighty.

It was said that if a compound gives a slowly-crystallizing deposit from colloid *or* gives crystals that dissolve slowly, the difference in toxicity may be large. But the tests showed that each of the compounds giving a large difference in toxicity had *both* these qualities. One was not enough. The reason why both should be necessary is not clear. It may be that this is not a general rule; one quality or the other, if extreme enough, might produce a large difference in toxicity.

The lipoid-solubility, or solubility in fats, is often said to be important in deciding the toxicity of a contact insecticide. The implication, sometimes stated directly, is that in a group of very similar compounds like close analogues of DDT the most soluble compounds are the best contact insecticides (Martin & Wain, 1944; Browning *et al.*, 1948; Skerrett & Woodcock, 1952). It is certainly not true with this group of DDT analogues. They are all fat-soluble, but there is no relation at all between the toxicity (of colloid) and solubility in olive oil, which is often taken as a convenient measure of solubility in body fat. The difference in toxicity between colloid and crystals is not related to fat-solubility either.

The reactions of the insects to different sizes of particle seem to support the idea that the first step in penetration is solution of poison in some solvent, presumably the cuticle wax. It may seem rather obvious that it is better to use colloid than crystals, and that the qualities making for efficiency are slow crystallization of super-cooled poison if it is applied as a colloid, and quick solution of crystals if the poison is applied as a solid. But the tests of DDT on warm and cool *O. surinamensis*, and of the other analogues, suggest that with this species the qualities that affect speed of solution do not decide speed of kill if the insects are kept warm after treatment; they are important only if the temperature is low. These qualities ought to apply to some extent to any stable contact insecticide and to almost any species of insect; this has still to be confirmed. The temperature at which they become important will not necessarily be the same for different species.

Crystallization of DDT can be prevented by mixing other compounds with it. This kind of non-crystalline DDT is more toxic than pure DDT in tests of dusts on sheep keds or vinegar flies (Riemschneider, 1950), and in tests of films on mosquitoes

or DDT-resistant houseflies (Ascher, Reuter & Levinson, 1951; Ascher & Reuter, 1953). In the film tests the non-crystalline DDT may stick better to the insects than crystals do, and for this reason may seem to be more toxic. But Beran (1952) found that impure non-crystalline DDT is more toxic than pure crystalline DDT when equal amounts are applied directly to houseflies. In all these tests the insects were kept warm (24–28° C.) after the poison was applied. From this it seems more likely that a low crystallization tendency is in general a helpful property at all temperatures and not just at low ones.

In practice it will seldom be possible to apply solid contact poisons in colloidal form. They are often formulated in one crystalline form or another. If a poison is to be efficient it must be able to saturate the cuticle wax quickly. This may be the result of its own properties, or of formulation; but in either case attention should be given to speed of solution rather than fat-solubility, which has perhaps been over-emphasized in the past. Some degree of fat-solubility is certainly necessary, but it need not be very high.

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## RECENT WORK ON MOLYBDENUM AND SOME MICRO-NUTRIENT INTERACTIONS

By

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### INTRODUCTION

Since 1941-42, when the effect of various incidental constituents of Chilean nitrate were being studied, micro-nutrient investigations in the Botany Department at Rothamsted have been largely concerned with molybdenum. This element is the most recent to be shown essential for higher plants, and not until 1939 did Arnon and Stout prove it essential for tomato. Piper (1940), Hoagland (1941) and Warington (1946) followed with oats, plum and lettuce respectively, and proof has now been extended to a number of other crops. It was from field experiments in Southern Australia and Tasmania, however, that the practical importance of the element first came to be appreciated, Anderson (1942) and Fricke (1943) demonstrating that failure of subterranean clover on certain ironstone soils was due to molybdenum deficiency. The discovery in New Zealand (Davies, 1945, and Mitchell, 1945) that whiptail disease of cauliflower was caused by molybdenum deficiency soon followed, confirmation of the field symptoms being obtained in sand culture by Hewitt and Jones (1947).

### MOLYBDENUM DEFICIENCY

Among the points established during the course of the earlier field trials was the greater availability of molybdenum under alkaline conditions (Stephens and Oertel, 1943) a fact to which Fricke (1944) attributed the benefit he obtained from the addition of lime only on soils responding to molybdenum dressings. These results fall into line with those of Ferguson, Lewis and Watson at Jealott's Hill (1940), who had found that liability to "teariness" in cattle, caused by excess molybdenum in the herbage of pastures in Somerset, was increased by a rise in soil pH.

The notable response of legumes to molybdenum and the observation that molybdenum-deficient clover was invariably pale, led to enquiries regarding the effect of this element on nitrogen fixation by the nodule organism. Bortels (1930) had already shown that molybdenum was needed for normal growth of *Azotobacter* in culture media lacking nitrogen, and Steinberg (1936) that it was required for nitrate reduction by *Aspergillus*. Jensen and Betty (1943) recorded increased nitrogen content in the roots of molybdenum-treated lucerne and white clover, and high concentrations of the element in their nodules, while in 1946 Anderson and Thomas followed with proof that molybdenum was essential for symbiotic nitrogen fixation. Mulder (1948) confirmed Steinberg's results and showed in addition that molybdenum was required for nitrate reduction in higher plants of a non-leguminous type and also for denitrification. Hewitt, Agarwala and Jones (1950) further found



that ascorbic acid production was much diminished by a lack of molybdenum.

Since the beneficial effect of liming suggested that response to molybdenum might be influenced by the calcium supply or the pH of the substrate, and one function of the element at least seemed to be connected with nitrogen nutrition, the next series of solution cultures at Rothamsted were designed to study the effect of these three factors (Warington, 1950). Here the amount of calcium supplied was found to have a marked effect on growth of both lettuce and red clover, more being required as acidity increased, but the level provided had no influence on the response of either plant to molybdenum. Variation in initial pH value (4.2–8.2), with calcium at a uniform standard rate, also affected growth very noticeably, in spite of a rapid levelling up in the reaction of the solutions, but with the possible exception of the most alkaline medium, visual molybdenum deficiency symptoms were invariably obtained unless molybdenum was provided. Response to molybdenum occurred with both species at all levels of nitrogen tried, and in both inoculated and uninoculated clover, the number of nodules formed in the latter set being greater when molybdenum was lacking, as described by Anderson and Thomas (1946). Nitrate-nitrogen accumulated in the shoots of the molybdenum-deficient lettuce and clover shoots confirming the results of Mulder (1948) and Hewitt, Jones and Williams (1949). There was also some indication that lettuce was more liable to damage from excess molybdenum when the nitrate supply was raised, an effect in keeping with the results of subsequent pot experiments (Brenchley, 1948).

#### MOLYBDENUM EXCESS

##### (a) *Microscopic effects*

Prior to the discovery that molybdenum was essential in plant nutrition, Sheffield (1934), working at Rothamsted, had found that addition of salts of molybdic acid induced changes in cell contents of solanaceous plants, inclusion bodies similar to those resulting from virus infection being formed. The nature of the compound, however, was not determined. Later microchemical tests, carried out on tissue from potato tubers and tomato shoots of plants grown with toxic quantities of molybdenum, showed that the characteristic golden colour developed under these conditions was caused by globular yellow bodies of a tannin-molybdenum complex (Warington, 1937). In the tomato, blue granular compounds of molybdenum with anthocyanin were also detected. A form of leaf mottling appeared on the leaves of tomatoes suffering from excess molybdenum, simulating virus symptoms, but subsequent inoculation tests showed that the plants were free from disease.

##### (b) *Macroscopic effects*

Most plants show high tolerance to molybdenum, and herbage containing amounts sufficient to cause "teart" disease of cattle remains undamaged itself. The species comprising the pastures, however, vary widely in their capacity to absorb the element, clovers and Yorkshire fog in particular showing much higher contents than the other grasses or weeds growing on the same soil

(Ferguson, Lewis and Watson, 1950). Tolerance to molybdenum also depends on the nature of the soil as well as the crop, Brenchley (1948) finding that dressings harmless to tomatoes grown on loam or allotment soil were very toxic on sandy Woburn soil. Further, *Solanum nodiflorum* was uninjured on allotment soil by a dressing which proved lethal to it on a cucumber soil rich in nitrogen, and while flax suffered considerable damage on this latter soil, tomato treated with the same rate of molybdenum on it remained unharmed. Other soil properties as well as nitrogen content and pH value would, therefore, seem to be factors determining uptake of molybdenum. HCl-soluble iron may also be of importance, for of thirteen Australian soils tested, Williams and Moore (1952) found least molybdenum absorbed by oats when the soil was rich in iron, the differences reaching significance independent of pH value.

#### INTERACTIONS BETWEEN MOLYBDENUM AND OTHER ELEMENTS

It is generally recognized that interaction between the various major and minor elements are of paramount importance, and much recent work with molybdenum has dealt with this aspect. From pot and field experiments (1948, 1949a), Millikan concluded that manganese and molybdenum were antagonistic, and showed later (1951) that addition of high concentrations of molybdenum to flax grown with excess manganese reduced the manganese content and altered its distribution, while Anderson and Spencer (1950) found that manganese accentuated molybdenum deficiency in clover and lowered its uptake.

Earlier Millikan (1947) had shown that molybdenum, if presented in sufficiently high concentrations, could counteract chlorosis induced in flax by a number of heavy metals given in toxic amounts. Hewitt (1949), on the other hand, found molybdenum enhanced the chlorotic symptoms of metal excess in sugar beet. He considered that some aspect of nitrogen nutrition was probably involved whichever way the interaction worked, and Bennett (1945) had already put forward the view that chlorosis was a disturbance of nitrogen as well as of iron metabolism.

The possibility that vanadium might give similar results to molybdenum seemed worth investigating, for Horner *et al.* (1942) had shown that the two elements could replace each other in *Azotobacter* nutrition, though Vanselow and Datta (1949) found no evidence for this in citrus. Comparison was, therefore, made of the effects of high concentrations of molybdenum or vanadium in the presence of manganese excess (Warrington, 1951). Flax and soybean were grown in nutrient solutions containing manganese at toxic (10–25 p.p.m.) and non-toxic (1 p.p.m.) levels, each combined with a range of concentrations of molybdenum or vanadium. Relatively high rates were required before any interaction with manganese was obtained, and the effects of the two elements were contrasting. Molybdenum (20 p.p.m. and to a less extent 10 p.p.m.) intensified the chlorosis induced by high manganese as Hewitt (1949) found with sugar beet, but both rates of molybdenum proved harmless in the presence of only 1 p.p.m. manganese.

Vanadium (equivalent to 1.0 or 5 or 10 p.p.m. Mo), on the other hand, counteracted some of the symptoms of manganese toxicity,

suppressing at least temporarily the apical chlorosis of both crops and reducing the leaf curling in soybean, though eventually the higher levels of vanadium induced apical chlorosis on their own account. Vanadium equivalent to only 0.1 p.p.m. Mo, however, failed to exert any noticeable effect at all. Thus, under this set of experimental conditions, high vanadium gave results similar to those obtained by Millikan for high molybdenum (1947) and later by him for aluminium also (1949b).

#### INTERACTIONS BETWEEN VARIOUS METALS AND IRON

##### (a) *Visual effects*

That metal toxicity causes disturbances in iron nutrition is no new discovery, for in 1919 Johnson cured pineapples suffering from excess manganese by spraying with iron. Similar antidoting effects of iron on other metals in excess have been found by various authors using either additions of iron to the nutrient medium or external applications to the leaves. Counteraction of metal toxicity by elements other than iron has, however, only recently been claimed. Since vanadium was one of the elements possessing this property, information regarding its effect on plants suffering from a direct (as distinct from metal-induced) shortage of iron seemed desirable. Ferric citrate was used as a source of iron, the standard amount selected as control depending on the crop grown. Within the concentrations tried (0.05–5 p.p.m. V) vanadium failed to relieve iron-deficiency chlorosis in soybean or flax, 2.5 or 5 p.p.m. V in fact proving more toxic if the iron content of the solution was reduced to one-half or one-third of the control (10 p.p.m. Fe) (Warington, 1954). Increasing the iron to 20 p.p.m., on the other hand, almost removed the symptoms of vanadium excess in peas in both root and shoot, and similar, though less-pronounced, effects were obtained with flax. Injury from manganese and molybdenum excess was similarly reduced by an increase in the iron provided, but if two or more of these elements were presented together the same quantity of iron was less efficient in counteracting their toxicity. This suggested that their effects towards iron were additive. The method of supplying the iron was important, for the same total amount given gradually proved less capable of offsetting the damage from vanadium and molybdenum than when supplied in a single initial dose. This, however, did not hold for manganese. Identical changes in the level of iron supplied had little or no effect in the presence of low concentrations of these elements, though there were indications, confirmed later, that there was a limit to the amount of iron that could be given without causing injury. Damage to citrus from excess iron has been described by Smith and Specht (1953), who, moreover, found it could be offset by application of high copper, zinc or manganese. A similar compensating effect of high molybdenum on excess iron has been found in flax (Warington, unpublished).

##### (b) *Effects on plant composition*

Attempts to interpret these interactions between manganese, molybdenum, vanadium and iron necessarily include a study of the changes in plant content of the elements concerned, though visual

differences may occur without any corresponding change in plant composition. Chlorosis, for example, is not always accompanied by a reduction in iron content of the shoot. McGeorge (1949) found a correlation between chlorosis and the soluble fraction of iron only, though Smith, Reuter and Specht (1950) showed that it held for total iron if the material was washed with a detergent. Absence of any correlation was interpreted by Millikan (1949a) as indicating a lack of utilization of iron within the plant rather than to a reduction in uptake. Analyses of soybean shoots (Warington, 1954) showed that the total iron content was scarcely affected by the quantity of iron supplied (5–20 p.p.m. Fe) provided manganese, molybdenum and vanadium were present at a low rate, but it was much reduced by high concentrations of all three elements. On the other hand, there was little change in the iron found in the shoots of flax grown with high vanadium. Berger and Gerloff (1947) and Sideris (1950) also record a drop in iron content of shoots of potato and pineapple respectively on the addition of high manganese. Sideris attributed this to interference with translocation and immobilization of iron in the root, as there was no indication of external precipitation, a view supported by Epstein and Stout's results (1951). Smith and Specht (1953) have described similar inhibitory effects on movement of iron within the plant following the addition of high copper or zinc.

The manganese and vanadium contents of the soybean shoots, already referred to, fell sharply as the iron supply was increased, in agreement with the results of Twyman (1951) and Morris and Pierre (1947) for manganese. Reduction in molybdenum content, however, was less clearly shown, in spite of the fact that visual toxic symptoms had been counteracted by the additional iron. The method whereby the iron offsets metal toxicity thus appears at first sight to be a reduction in the amount of injurious metal in the shoot. This does not explain the recovery of colour following the application of iron paints or sprays, and it seems more likely that some interaction between the heavy metal and iron takes place in the root, resulting in changes in translocation of both iron and metal. Further analytical work will be needed before any definite conclusions can be drawn.

#### OTHER FACTORS AFFECTING METAL TOXICITY

The degree of injury caused by molybdenum, manganese and other elements also depends on the nature of the nitrogen supply. Millikan (1950) found manganese more toxic with nitrogen supplied as nitrate than as ammonia; the reverse was true for molybdenum. Further, ammonium molybdate proved more toxic than the sodium salt in the presence of nitrate, though both were equally damaging if the nitrogen was given in the form of ammonia or urea. Response to iron was also influenced by the form in which the molybdenum was provided, ammonium molybdate proving more efficient in overcoming iron deficiency than the sodium salt (Millikan, 1950; Warington, unpublished). That the incidence of chlorosis is affected by many other factors such as potash supply, light, temperature, age of plant, etc., only adds to the complexity of the problem.

## CONCLUSIONS

The ultimate aim of all investigations with micro-nutrients is to determine their function in plant nutrition. Information on this point is at present scanty. Approached from the point of view of deficiency, manganese, molybdenum, copper and zinc each appear to be associated with specific plant processes. If given in excess, these four elements may either cause disturbances in iron nutrition similar to each other and to those induced by metals not yet considered essential, (e.g., vanadium, nickel and cobalt) or exhibit antagonistic properties according to circumstances. The health of the plant seems to depend as much on a correct balance between the nutritive elements as on the presence of each, and precise statements regarding demand or tolerance for any particular micro-nutrient are, in consequence, of limited value only. Much further work will be needed before these metal interrelationships are fully understood.

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