PHYTOTOXICITY*

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THE role of chemicals in altering metabolism at critical biological sites is a current segment of research in the study of plant chemotherapy. The implied concept of phytotoxicity needs hardly any justification since the success of any chemotherapeutant rests on the selective toxicity of the chemical or, any products of its conversion to the pathogen, the ease of its absorption and translocation and, more important of all, its freedom from causing inhibitory effects on plant growth. Although a wealth of literature exists on the application and effectiveness of a variety of chemicals and their selective toxicity to macro and micro-organisms in pest and disease control,[1-3] there is urgent need for fundamental information on the alterations induced at cellular and subcellular sites on the inhibition so often noticed in plant vigour and growth following a chemical treatment. Many pesticide, insecticide and fungicide schedules are now known to alter soil-plant rhizosphere interactions,[4] membrane permeability, translocatory and transpiratory processes, induce changes in oxygen and ion uptake, intrude on enzyme-substrate-energy involving reactions, affect photosynthesis, nucleic acid and protein synthesis. It is, therefore, obvious that studies on crop physiology and factors governing the role of individual chemicals and groups of compounds used in soil or spray schedules on plants are essential to properly evaluate phytotoxic effects which may vary with the habitat, meteorological and soil conditions. These aspects are, therefore, discussed here in the context of the growing necessity for introspection in crop management practices with particular reference to the use of proprietary fungicides.

Recent experiments on Flax[5] with the triethanolamine salts of 2 : 4-dichloro-, 2 : 4 : 5 trichloro-, and 2-methyl-4-chlorophenoxy acetic acids (which are freely translocated when sprayed on leaves or applied in droplets on cotyledons) have shown marked inhibition of growth rates with increasing concentration of application. The order of inhibition due to these substituted phenoxy acetic acids was shown to depend on the absolute amount deposited on the shoots and that overall phytotoxic effects are dependent on the type of compound. Work with shoots of peas[6] has shown that these are readily damaged when high Monuron [3-(p-chlorophenyl)-1, 1-dimethylurea] concentrations were applied for even short periods of time especially under conditions of high metabolic activity. Pronounced growth inhibition in tomato, cucumber, strawberry and carrots due to soil application of insecticides such as DDT, Dieldrin and Aldrin have been reported.[7] Chlorinated hydrocarbon insecticides such as these are frequently used to control soil-borne pests of horticultural and agricultural crops. When applied frequently these very stable and persistent chemicals may eventually accumulate in many soils. The residual effects of these on plants grown in treated soils is little studied, despite observations that they can seriously injure growth.[8-15] Studies on alkyl and aryl substituted ureas and thioureas[6] whose toxicity to peas was shown to be a function of their solubility in water have indicated the possibility that these chemicals may affect enzymatic processes by distortion of sites at which integrated reactions occur in cells. In discussing the toxicities of physical poisons such as the ureas substituted with acidic or basic groups and related compounds, Hassall[6] pointed out that growth inhibition stems mainly from changes in lipoprotein phase boundaries in cells whether the site of action is the chloroplast, the mitochondrion or the cell membrane. Coumarin, whose phytocidal properties have been described by Audus and Quastel[16] was shown to inhibit water uptake by altering the permeability of the protoplasm in embryonal cells in wheat seed.[17] Similarly, loss in permeability of tissues with toxaemia has also been reported from this laboratory[18] with fusariose cottons. This vivotoxin, formed *in situ*, and highly stable in the rhizosphere of crop plants can impair membrane permeability at even very low concentrations (10^{-9} to 10^{-8} M). Its preferential avidity for Fe may also cause the removal of this metal from porphyrin moieties as was shown here in *in vivo* studies.[19] These primary changes in osmoregulation of host cells following toxæmia is also evidenced by an increased conductivity and accumulation of electrolytes in cotton plants.[20] Further, spectrochemical analyses clearly indicated ionic

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imbalance in infected leaves, increased tissue contents of Mg, Ca and Mn[21] and pronounced increase in oxygen uptake.[22]

Studies on the passage of fungicides through the relatively complex chemical and osmotic barriers in higher plant tissues have also shown that active transport across cells is influenced by the type of chemical, the solvent used, dosage, area of application and the relative humidity. Experiments with a new class of fungicides belonging to the class of β-amino-ethyl-aril-ketones on bean and tobacco have shown that penetration through roots and stems and translocation to leaves of C^{14} labelled substances occurs relatively quickly.[23] Although the potential usefulness of this in the control of bean rust caused by *Uromyces* spp. was considered by them as obvious, it was pointed out that systemic migration of these compounds occurs not only into a restricted area of the treated leaf but also into the upper parts of the plant as shown by autoradiography of treated plants 3 days after application of S 210-C^{14} in aqueous solution. In leaves, passive diffusion through cuticle and epidermis into different areas of palisade and spongy mesophyll also occur independently of the vascular mass flow. This idea of the non-specific permeation mechanism which may depend on the solubility of substances in membrane components of cells stimulates thought on the possible presence and location or absence of sensitive reaction sites through which an agent can cause phytotoxic effects in inhibiting normal plant growth. An example of this is afforded by the mechanism of action of chloramphenicol. This lipid-soluble antibiotic, produced by species of *Streptomyces venezuelæ*, is generally absorbed by plant roots and translocated to leaves.[24] It inhibits protein synthesis in bacteria but not fermentation, respiration or nucleic acid synthesis.[25,26] At concentrations ten times higher than are effective in bacteria (200 μg./ml.) protein synthesis in cell-free preparations of particles from higher plants is sensitive to chloramphenicol.[27] At still higher concentrations (1000 to 2000 μg./ml.) it inhibits salt uptake by red beet slices and carrot root tissue without significantly altering respiration.[28] Phosphorylation and respiration are not affected by chloramphenicol in normal mitochondrial preparations,[29] and as shown from the work of Webster[30] and Jacoby and Sutcliffe[31] on carrot slices, it has been assumed that chloramphenicol blocks the incorporation of amino-acids into nucleoproteins. Steward and Miller[32] earlier reported on a close relationship between the process of salt accumulation and protein synthesis. Uhler and Russell[33] working on barley have shown that chloramphenicol retarded the accumulation of Ca and Ru significantly although transpiration was little affected. Jacoby and Sutcliffe[31] suggest that the incorporation of amino-acids into proteins proceeds by at least two mechanisms in carrots and that one of these is chloramphenicol-sensitive and related to protein synthesis while the other unrelated to protein synthesis is insensitive to the action of this drug. It then became necessary to clarify the effect of chloramphenicol on inorganic ion absorption and to elucidate the relation between salt absorption and protein synthesis. Thus, studies on phytotoxicity of chloramphenicol, long known to be an inhibitor of protein synthesis, have paved the way to its implication in processes affecting salt uptake and the mechanisms of active transfer which convey ions across root membranes.

Permeability changes such as those described here can have far-reaching effects on soil plant relationships especially in tropical soils which differ from temperate ones in their ability to hold and supply nutrients.[34] Even on the broadest basis soils in many areas of the tropics are subject to more intense weathering processes and leaching of plant nutrients. Unproductive plant growth induced by pesticidal and fungicidal schedules are, therefore, naturally to be viewed with concern. Quite obviously, indiscriminate chemical schedule might do great harm if a move is made to copy soil or spray practices of chemical toxicants evolved in temperate environs for a totally different tropical climate where rapid translocatory, photosynthetic and, in general, accelerated metabolic rates prevail more commonly in plants subjected to high temperature variations. Little is known about the toxicities of recalcitrant insecticide, herbicide and fungicide molecules whose persistence and accumulation in soils from frequent application may eventually assume phytocidal proportions.

Several herbicides such as 2, 4-D (2, 4-dichlorophenoxyacetic acid), Dalapon (2, 2-dichloropropionic acid), TCA (trichloracetic acid) can persist for 2 to 8 weeks while Monuron [3-(*p*-chlorophenyl)-1, 1-dimethyl urea], 2, 4, 5–T (2, 4, 5-trichlorophenoxyacetic acid persist for 4 months to almost one year. Insecticides such as benzene hexachloride and chlordane are known to persist for more than ten years. *Ethylene dibromide, dichloropropene-dichloropropane* mixture and dibromochloropropane are

often applied as nematocides. Chloropicrin and methylbromide are used both as nematocides and fungicides. Formaldehyde, Captan (N-trichloromethyl thio-1, 2, 3, 4-tetrahydro thalimide), Thiram (tetramethylthiurum disulphide), Nabam (disodium ethylenebis dithiocarbamate) and other dithiocarbamates such as Ziram, Maneb, Ferbam, chlorinated quinones like Chloranil (Spergon), Dichlone (Phygon) are used as fungicides. Very little is known concerning the microbiological detoxication of many of these fungicides and insecticides.[35]

The degree and duration of phytotoxicity with herbicides such as the phenoxyalkyl-carboxylic acids, substituted ureas, nitrophenols, chlorinated acetic and propionic acids, phenyl-carbamates and thiocarbamates used in weed control, turf and crop management is not known.[35]

Pesticides, many of which are not easily decomposed in soil can act on the saprophytic soil colonizers and prove inimical to plant growth. Seed dressings with organo-mercurials and compounds that inhibit fungal growth can prevent the normal colonization of nitrogen-fixing bacteria in the legume rhizosphere. DDT, BHC, Chlordane, Aldrin, Dieldrin, Parathione and Toxaphene may affect nitrification and legume nodule formation. Thiram, Diclone, Chloranil and Captan are toxic to rhizobia. Chloropicrin interferes with ammonification.[35]

It is, therefore, hardly possible to overlook the ability of these antimicrobial agents for they can inhibit biological systems of one kind or another.

Work in this laboratory on the effects of 8-hydroxyquinoline, EDTA, Thiourea, *p*-Nitrophenol, Sulphanilamide and Griseofulvin on rice seedlings showed that these are phytotoxic to rice.[36] Sulphanilamide and Griseofulvin induced characteristic alterations in the soluble nitrogen compounds of varieties of *Oryza sativa* susceptible and resistant to helminthosporioses.

Results of many metabolic investigations indicate a relationship between changed water relations and alterations in metabolic processes following fungicide treatment. Sodium trichloracetic acid was shown to inhibit both respiration and photosynthesis in tomatoes.[37] Studies with three new compounds E 52, E 54 and CH showed blocking of respiration and inhibition of photosynthesis to varying degrees.[37] Substituted ureas and carbamates at concentrations lower than those affecting cell division suppressed photolytic reaction in isolated chloroplasts.[6] Urea, Ethylurea, Monuron, Thiourea, Ethylthiourea and Ethylcarbamates act as physical poisons with varied toxic effects on living organisms.[6] Fungicides such as Cycloheximide, Dyrene and Zineb affect the synthesis of chlorophyll, deoxyribonucleic acid and ribonucleic acid.[38] Phytotoxicity due to Cycloheximide was shown to be more severe on well-grown rather than on poorly-grown onion plants. Dyrene in addition induced decreased levels of DNA in leaves of onions. While DNA synthesis was inhibited by these fungicide sprays RNA synthesis was increased abnormally following sprays with Cycloheximide. The decrease in chlorophyll content was more marked in younger leaves than in older ones. Although there were no differences in dry matter, ash and water contents of treated and control leaves, fungicides clearly influenced the synthesis of DNA and RNA. Similarly, the antiblast antibiotic Blasticidin S was shown to cause a marked accumulation of RNA in yeast even at as low a concentration as 1 p.p.m.[39] At excessive concentrations (40 μg./ml.) Blasticidin was phytotoxic to rice, soybean, apple, pear. peach, cabbage, cucumber, tomato and other plants. Inhibition of cell division by 6-methyl purine was shown to occur *in vitro* in pith and callus cells even at 10^{-9} M, while complete inhibition of cell division accrued with 10^{-6} M.[40] Aflatoxins produced by moulds (*Aspergillus* spp.) similarly suppress cell division and the synthesis of even the genetic material DNA.[41]

A curious sidelight of the effects of these toxins has recently come to light on studies with the aflatoxins produced by common species of Aspergilli. The generally observed non-fatal but weakening and debilitating effects which are generally considered inevitable due to living conditions in the tropics leading to the often prevalent lassitude of tropical man is now really considered simply an effect of eating mouldy foodstuffs containing these aflatoxins.

Indeed, the problem of phytotoxicity needs tackling not only in the area of alterations in metabolic patterns in plants under chemotherapeutic stress but also on the role of mould-produced poisons as well in abnormal growth.

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