Selectivity of Fungicides

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The concept of selectivity is ancient. It is easily extendable to cover a primary aspect of fungicidal action. Like many ancient concepts, this one finds pithy expression in a proverb, "One man's food is another man's poison."

When we use the term, selectivity, we mean to use it in the sense of a selective weedkiller. 2,4-D is a selective weedkiller. It kills dandelions in the lawn, not the bluegrass. We use it in the sense of natural selection. Drought-resistant plants survive in the arid regions of the world, while other plants die. A desert is selective in that sense.

We do not mean to insert a teliological slant into the discussion. We do not assume that a selective fungicide expresses any volition.

We must emphasize that selectivity is an abstract term that may be likened to a street that carries traffic in both directions. We have selective organisms as well as selective chemicals. A man selects his food and rejects his poisons. In this case a poison is selective for one man, not for the other.

In logic the matter is equivalent to that for the host-parasite complex. The host "selects" its parasites; the parasite selects its hosts. And neither can be investigated without the other.

In this paper we shall undertake to synthesize our experiences and those of other plant pathologists with fungicidal selectivity into a set of principles as they look to us. Some of the points are as old as Methuselah. Some are newer. We sometimes think, for example, that selectivity due to acquired resistance is a new idea as dramatized by the penicillin-resistant *Staphylococcus* that harrasses the hospitals or by the diphenyl-resistant strains of organisms that rot oranges in transit. In fact this idea is old—witness the case of old Mithridates who made himself resistant to arsenic and other poisons by eating slightly increasing daily doses for a long time.

We suggest that selectivity can be presented in six parts: (1) selectivity between fungus and host; (2) selectivity between disease and disease; (3) selectivity between fungus and fungus; (4) selectivity between strain and strain; (5) selectivity between response and response; and (6) selectivity between analogue and analogue.

Reviews Are Few

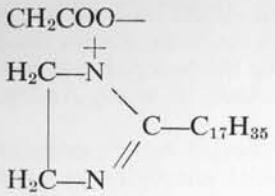
In this age of reviews, perspectives, advances, and reviews of reviews, it is astonishing that this subject has almost wholly escaped direct review. In a sense, of course, any treatment of fungicidal action deals with selectivity, but selectivity tends to be implicit more than explicit in such treat-

ments of the subject. Somers (82) has recently essayed a very short treatment of specificity. Kreutzer (49) has discussed selectivity of soil fungicides. Ours will be much more inclusive. This paper is an expanded account of a paper we gave at the International Botanical Congress in 1964 (39).

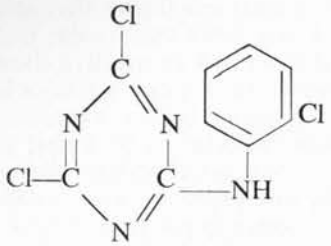
SELECTIVITY BETWEEN FUNGUS AND HOST

Selectivity between fungus and host is an old principle that goes back at least to Ehrlich—the *dosis tolerata* and the *dosis curativa*. Ehrlich called this the chemotherapeutic index. Sulfur, the ancient old fungicide, is tolerated by the Rosaceae, but not by Cucurbitaceae. Therefore, it is used on fruits, not on cucumbers. Bordeaux mixture is not tolerated on Rosaceae and is not used on that family. It *is* tolerated on Solanaceae and *is* used on that family.

The relative safety of bordeaux and sulfur on green plants is due to the cuticle barrier. Homologues of glyodin (I) (i.e. 2-imidazoline) are interesting here. Lengthening the carbon tail attached to the number 2-carbon increases the permeability and increases both the fungitoxicity and phytotoxicity. According to Wellman and McCallan (96), phytotoxicity reaches a maximum at 11 carbons. As the hydrocarbon tail is further lengthened, the permeation into foliage declines and host damage declines. But permeation into the fungus continues to rise and fungitoxicity rises until there are 17 carbons in the chain.



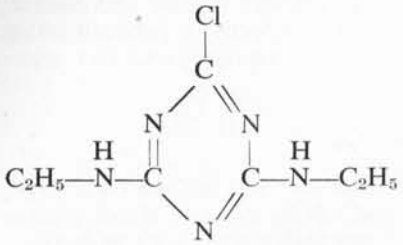
I. Glyodin



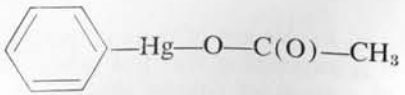
II. Dyrene

Hence, commercial glyodin is the 17-carbon compound. It is the most fungitoxic, the least phytotoxic.

Then we have the striking selectivity of the *s*-triazines. Dyrene (II) is a fungicide; simazine (III) is a weedkiller. Simazine can never be a foliage fungicide. It has no safe chemotherapeutic index.



III. Simazine



IV. Phenylmercuric acetate

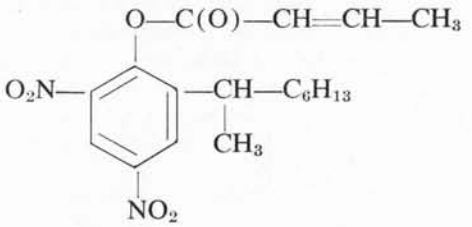
Similarly, phenylmercury acetate (IV) is a foliage fungicide for different kinds of foliage but a weedkiller for crabgrass.

2,4-D exercises an inverted selectivity. It kills green plants, but has essentially no fungitoxicity. It kills the host, but allows the fungus to survive.

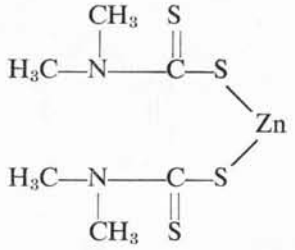
SELECTIVITY BETWEEN DISEASE AND DISEASE

To some extent selective action between disease and disease is related to the selective action between fungus and fungus to be discussed later. We shall consider here some special cases.

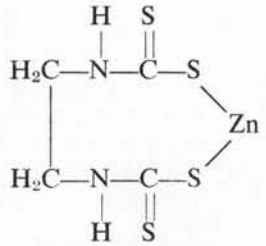
We have known for decades that sulfur is selective for powdery mildews and not for downy mildews—that bordeaux mixture is selective for downy mildews and not for powdery mildews. Karathane (V) (74) is a modern discovery for powdery mildews (102) but it is poor on most foliage diseases (Doling and Hepple, 14).



V. Karathane



VI. Ziram



VII. Zineb

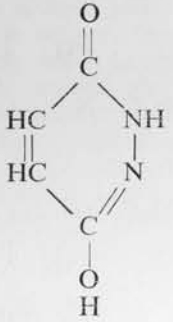
Another interesting case of selectivity is to be seen in the dithiocarbamates. Ziram (VI) controls alternarial blight of potato reasonably well, but not phytophthoral blight. Zineb (VII) another dithiocarbamate, controls both diseases (37).

Fungicides That Worsen Some Diseases

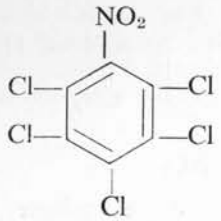
The fascinating feature of selectivity among diseases is that some perfectly good fungicides, while controlling one disease very well, will worsen another. I (J.G.H.) saw this first 10 years ago in Switzerland. Zineb was controlling *Plasmopara* on grapes, but it was worsening powdery mildew on the same vines and *Botrytis* on the grapes they bore. On apple,

captan controls scab and worsens powdery mildew. Zineb controls diseases on the foliage of tomato but worsens *Botrytis* rot on the fruit (12).

The explanation seems to lie in the host response, not in the fungus. Barner and Roder (3) say that zineb induces a thin cuticle and, thus, increases susceptibility to powdery mildew. There is a little evidence that dithiocarbamates elevate the sugar content of tissues and make them susceptible to powdery mildew or *Botrytis* (38). This sugar theory can be checked out with maleic hydrazide (VIII)—the growth substance. This compound damages phloem. As a result sugar piles up in the leaves above the damaged area and disappears from the roots below it. Therefore, maleic hydrazide increases a high-sugar disease like powdery mildew in the leaves (61), and increases a low-sugar disease like helminthosporial rot on the root of barley (79). By the same token DDT increases the leaf sugar in wheat and increases the rust, which is another high-sugar disease (21).



VIII. Maleic hydrazide



IX. Pentachloronitrobenzene (PCNB)

Sometimes fungicides increase a second disease by killing off the competing organisms. Gibson (28) and Erwin *et al.* (19) say that organic mercury increases pythial attack on cotton seedlings by killing off the anti-pythial flora in the soil. They did not complete the required proof for this explanation by showing that *Pythium* is resistant to the mercury, however. This seems doubtful (See Ref. 2).

Sometimes fungicides increase a second disease by encouraging its complementary organisms. Although pentachloronitrobenzene (PCNB, IX) inhibits rhizoctonial attack, Rich and Miller (78) have reported that it greatly increases verticillial wilt on strawberries by increasing nematodes in the soil which wound the tissue and open the way for *Verticillium* which has not been killed by the PCNB.

SELECTIVITY BETWEEN FUNGUS AND FUNGUS

Most of the classical fungicides like copper, mercury, phenols, creosote, captan, and zineb are wide spectrum compounds. Therefore, these compounds show little selectivity. They probably react with numerous biochemical systems in the cell.

We are here concerned, of course, with narrow spectrum compounds, because that is what selectivity means. In our laboratory we have made

extensive studies of two test organisms and the selective action of fungicides on them in the laboratory. The organisms are *Monilinia fructicola* and *Stemphylium sarcinaeforme*. With the reader's permission we will discuss what we have learned with these and from related data in the literature.

We shall be concerned with selectivity due to habitat, to permeation, to detoxication, and to acquired resistance.

Selectivity Due to Habitat

Monilinia normally lives in the acid habitat of a ripening stone fruit. Even in culture it creates an acid habitat by excreting acid into the medium. On the other hand, *Stemphylium* normally lives in the near alkaline habitat of a clover leaf. It secretes no acid. Therefore, *Monilinia* is not susceptible to acidic compounds, *Stemphylium* is.

Selectivity Due to Permeation

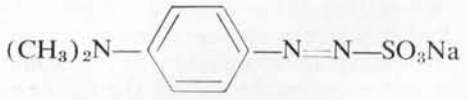
Monilinia lives on a high sugar diet of the stone fruit. *Stemphylium* lives on a low sugar diet of a clover leaf (101). *Monilinia* cells are full of fat. *Stemphylium* cells seem to have less. We assume that these differences account for the striking differences in the effect of fat soluble molecules. In general the fat solubility of a compound that is toxic to *Monilinia* must be much higher than that for *Stemphylium*.

This means, for example, that an aliphatic tail (haptophore) must be longer for maximum effect on *Monilinia* than for *Stemphylium*.

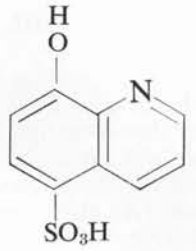
We have reported this effect for nicotinic acid esters (41), for dithiocarbamic acid esters (77), and for dioxanes and dioxolanes (40). We assume that these differences in lipid solubility are due to differences in permeation (75).

A striking example of the same situation concerns the differential effect of dexon (X) on *Pythium* and *Rhizoctonia*. It kills *Pythium*, not *Rhizoctonia* as Leach *et al.* (52) have shown. The sulfonic acid moiety in this compound makes it water soluble. Now *Pythium* is a water mold; *Rhizoctonia* is a dry-land organism.

In our studies of fungicidal action on our two test organisms, *Stemphylium* and *Monilinia*, we have been discouraged from seeking activity by adding sulfonic acid groupings. We have tested dozens. All seemed to weaken the compound. For example, in our tests oxine (41) is a reasonably fungitoxic compound; oxine-5-sulfonic acid (XI) is hardly more toxic to *Monilinia* than distilled water. We have always assumed that the deleterious action of the sulfonic acid group was to make the oxine so water soluble that it would not penetrate the tissues.



X. Dexon



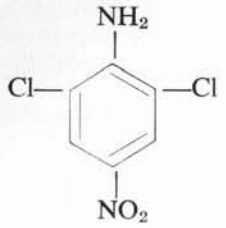
XI. Oxine-5-sulfonic acid

Dexon is different. Here a surfonic acid group seems to make it go. The organism that selects it is *Pythium,* however, not *Stemphylium* or *Monilinia* or even *Rhizoctonia.* We assume then that water soluble dexon permeates into a water mold and kills it, but it cannot permeate well enough into *Rhizoctonia* to kill it. We suspect that our bland compound oxine-5-sulfonic acid might also be specific for *Pythium.* In fact one wonders if *Pythium* might not be a good screening organism for water soluble types of fungicides.

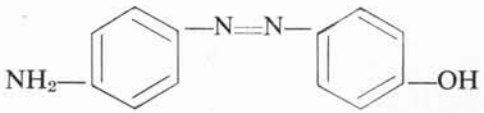
More evidence for this conclusion about differential permeation derives from Eckert's (16) study of the differential action of chlorinated nitrobenzenes (IX) on *Pythium* and *Rhizoctonia.* On the basis of the conclusion on dexon, one would expect that since chlorination of nitrobenzene increases fat solubility, it should improve potency on *Rhizoctonia* and reduce or not affect the potency of *Pythium,* and this is precisely what Eckert's data show.

The selectivity of *Pythium* for dexon and the exclusion by *Rhizoctonia* gave Garren (26) an opportunity to diagnose a puzzling disease of peanut. He could not be sure of distinguishing between *Pythium* and *Rhizoctonia.* He tested dexon. The disease was controlled. He concluded that *Pythium* was the cause.

Now, Ogawa *et al.* (64) showed that *Rhizopus stolonifera* is more sensitive to 2,6-dichloro-4-nitroaniline (XII) than *R. arrhizus.* We would hazard a guess that this is due to differential permeability.



XII. 2,6-Dichloro-4-nitro-aniline



XIII. 4-Amino-4′-hydroxyazobenzene

The data of Fox *et al.* (23) fit into the permeation niche. They discovered that 4-amino-4′-hydroxyazobenzene (XIII) is highly effective on *Pythium* not on *Rhizoctonia,* that chlorinating either ring, or substituting either the 4-amino group or the 4′-hydroxy group reduces the activity on *Pythium* (no data on *Rhizoctonia*). Either treatment of the molecule would serve to increase fat solubility and presumably reduce the permeation into *Pythium,* the water mold.

Selectivity Due to Detoxication

Numerous cases of selectivity are due to detoxication of the compound. To return to *Stemphylium* and *Monilinia,* the former is black because phenol oxidase is a terminal oxidase and it forms melanin from phenols. The latter fungus is hyaline and does not have a phenol oxidase as a terminal oxidase. On that account, as we have reported (76), *Stemphylium* detoxifies numerous phenols into melanoid pigments and is resistant to them. *Monilinia* cannot and it is susceptible to them.

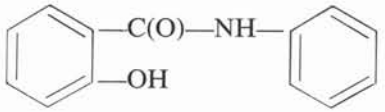
Hormodendron resinae is an extraordinary fungus because it can degrade creosote, of all things, and use it for food (57). Similarly, *Aspergillus flavus* can use salicylanilide (XIV) for food (47). To advert briefly to dexon (X), Tolmsoff (88) says that *Rhizoctonia* can degrade it; *Pythium* cannot.

According to Challenger (10) *Aspergillus sydowi* inactivates arsenic compounds by converting the arsenic to trimethyl arsine which escapes as a gas. The organism derives the required methyl groups from methionine.

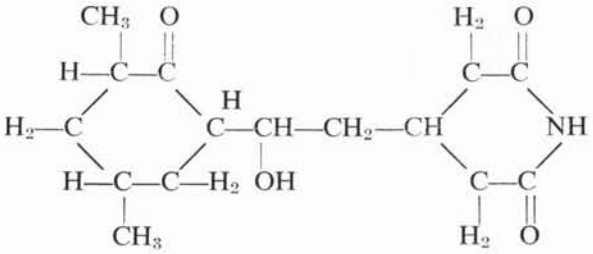
Resistance of *Aspergillus niger* to mercurial fungicides is attributed to precipitation of mercury by the large pool of cellular thiols which the fungus contains (2). *Rhizoctonia solani* and *Pythium ultimum* having little or no measurable free thiol pool are susceptible to mercurial fungicides.

Myrothecium verrucaria can degrade actidione (XV) (94) and *Trichoderma viride* can remove the chlorine from monochloracetate (48).

Woodcock (100) has reviewed a portion of the literature on detoxication.



XIV. Salicylanilide



XV. Actidione

SELECTIVITY BETWEEN STRAIN AND STRAIN

Natural Differences Between Strains

A large literature is available on the occurrence in nature of resistant strains of fungal species. For example, Fergus (20) says that his Canadian strain of *Ascocybe grovesii* is more resistant to actidione (XV) than his Colorado strain and Krstic (50) says that American strains of *Lenzites*

saepiaria are more resistant to *p*-methoxytetrachlorophenol (XIII) than his Jugoslavian strains. An interesting case of strain difference is reported by Shatla and Sinclair (80). Strains of *Rhizoctonia* that are resistant to pentachloronitrobenzene (IX) are also more pathogenic to plants.

Acquired Resistance Between Strains

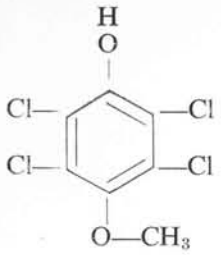
Another aspect of selectivity is acquired resistance to fungicides. This is commonly a practical problem for bactericides and insecticides, but it has not yet become a serious problem in plant pathology except possibly in the case of diphenyl (XVIII) for rot diseases of ripe orange. Both *Diplodia* (53) and *Penicillium* (34) have acquired resistance in nature.

There is a fairly large literature on acquired resistance in the laboratory to other benzene derivatives: trichloronitrobenzene (60), pentachloronitrobenzene (IX) (18), acenaphthene (98), 2,6-dichloro-4-nitroaniline (XII) (95). Resistance has been developed in numerous organisms in culture to metal fungicides like copper and mercury. This is reviewed by Ashida (1). Captan (XIX) seems to induce resistance in culture (67). Apparently the dithiocarbamates like zineb (VII) are recalcitrant. They do not induce resistance.

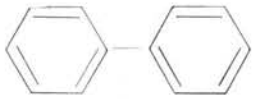
Our general assumption is that fungi probably develop resistance to compounds that react with a limited number of sites in the cell and that they do not develop resistance to compounds that can inhibit at several possible sites.

$$Cl—CH_2—COOH$$

XVI. Monochloroacetic acid



XVII. *p*-Methoxytetrachlorophenol



XVIII. Diphenyl

SELECTIVITY BETWEEN RESPONSE AND RESPONSE

We offer in this section some cases of selectivity in spore germination, growth, respiration, hyphal deformities, and sporulation.

The Growth Response

In discussing this class of selectivity we must generally relate the selectivity to growth, i.e., how are other responses affected differently from growth? It almost goes without saying, of course, that fungicides act selectively on growth. The reference above that dexon (Leach *et al.* (52)

reduces growth of *Pythium* and not growth of *Rhizoctonia* is a case in point. This is beautiful specificity. And, of course, as shown in a later section, different analogues of a compound give selective growth responses.

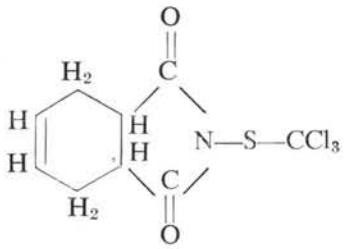
The Spore Germination Response

The question here is, do compounds affect growth and spore germination selectively? The answer comes back, "Yes!" Growth and germination are the responses often used in screening candidate fungicides. We tested approximately 1,000 random compounds in our collection on both responses using *M. fructicola* (42). We found that 5 per cent of the compounds inhibited growth more than germination, 18 per cent inhibited germination more than growth, 24 per cent inhibited both about equally and 53 per cent inhibited neither.

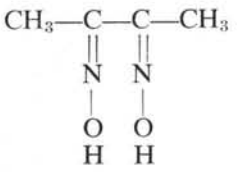
The larger percentage that inhibited growth selectively may be explained by the research of McCallan and Miller (58) that spores accumulate fungicides more rapidly than do mycelia.

If we consider types of compounds that inhibit growth and not germination in our test, we find that these are mitotic poisons like urethane, or fat solvents like benzene, acenaphthene, diphenyl (XVIII), γ-hexachlorocyclohexane. Rich (71, 72) showed that nitrobenzenes are in this class as well as certain chelators like dimethylglyoxime (XX) and α-furildioxime. Tetrachloroethylene is put in this group by Oyer (65), diphenyl by McCallan and Miller (58), and 4-chloro-3,5-dimethylphenoxymethanol by Dimond and Chapman (13).

The following compounds have been listed in the literature as affecting germination more than growth: aconitic acid (37), benzoic acid, methyl-*p*-hydroxybenzoate, Na-pentachlorophenate, methoxyphenoxyacetic acid, and dehydroacetic acid (6), oxine, captan, and TMTD (tetramethylthiuramdisulfide) by Domsch (15), and several antibiotics by Muller (62).



XIX. Captan



XX. Dimethylglyoxime

The Respiration Response

Not very much information is available on the differential effects of compounds on respiration versus growth or respiration versus spore germination. Cochrane (11) did show a qualitative difference between the respiration of spores and mycelium and McCallan and Miller (58) a quantitative difference. Germinating spores use more oxygen and for different purposes from growing hyphae.

In general the respiration data on fungicides cluster chiefly into two groups: (a) that spore germination is more sensitive to poisoning than

spore respiration (29, 59, 93, 58); and (b) that hyphal growth is more sensitive to poisoning than hyphal respiration (90, 55, 89).

What this means is that, in general, poisoned spores or hyphae will continue to use up oxygen after they can no longer grow. Walker and Smith (94) and Walker (93) hold that actidione is an exception, that spores will grow when no oxygen is being absorbed. This unusual conclusion is contraindicated by McCallan and Miller (58) whose data show that actidione fits the general picture.

Obviously, these data need clarification.

Until the problem is solved we will stay with the more general and easily defensible position that fungal tissue, whether spores or hyphae, can respire when it can no longer grow. In this case we can agree with Van der Kerk and Klöpping (90) that the compounds that fit here, and most do, are not acting primarily on the respiration system that involves O_2 consumption.

So far as differential effect on respiration between spores and hyphae is concerned, the data are few and mixed, and the effect seems to vary between compounds and between organisms. McCallan *et al.* (59) and McCallan and Miller (58) found hyphal respiration is more sensitive than conidial respiration to most fungitoxicants which they tested. Walker (93) puts phenols in this class. However, actidione affects spore respiration more than hyphal respiration (94, 95, 58). Walker puts all tested compounds other than phenols in the class with actidione.

The Monstrosity Response

Fungicides selectively produce monstrosities in fungi. The fungicide that did most to open our eyes to this phenomenon was griseofulvin, the curling factor for *Botrytis cinerea* first reported by Brian *et al.* (9) and reviewed recently by Grove (32). This compound causes the tips of germinating spores to swell, the tubes to grow curled, distorted, and much branched.

Horsfall (37) traces the subject of monstrosities for its first 10 years. By now a large number of compounds are known that produce curled, branched, bloated, or bursted fungal cells. We shall discuss these in terms of their possible action.

Clearly, the bloating deformity is due to some effect on the retaining structure, the wall. Either the compound weakens the wall and the normal internal stresses produce bulges that may or may not eventuate into excess branching, or the compound increases internal stresses so that a normal wall is stretched and bloated.

Suppose we discuss the wall first. Among other things it is comprised of chitin and cellulose.

Chitin insertion into the cell wall. The generally accepted theory for the action of griseofulvin is that the compound interferes with the deposition of chitin into the cell wall. It seems to exert no effect on nonchitinous fungi (63). As far as we know, no one has tested the effect of other bloat-inducing compounds on chitin emplacement, however.

Glucose insertion into cell wall. Fifteen years ago Tatum *et al.* (84) discovered that sorbose (XXI) produces mycelial overgrowths and distortions

in *Neurospora*. Recently, de Terra and Tatum (85) have shown that the glucosamine/glucose ratio in the hydrolyzates of the wall is increased. That presumably means that the cellulose part of the wall is reduced and the wall is weakened. Sorbose in an analogue of glucose (XXII). Presumably, sorbose interferes with the deposition of cellulose in the wall. One wonders if sorbose would inhibit cellulose decomposition.

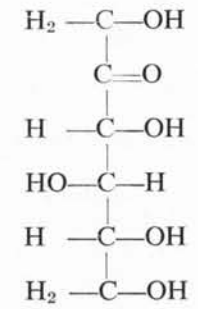
Suppose we go next to possible membrane alterations. If the semipermeable membrane is altered, water could enter more readily and burst the normal wall.

Entry of water into the cell. Surfactants do alter the membrane. Tatum *et al.* (84) say that they induce bloating. Manten *et al.* (56), Gornitz and Harnock (31) confirm this. Presumably, they allow excess water to enter and increase swelling.

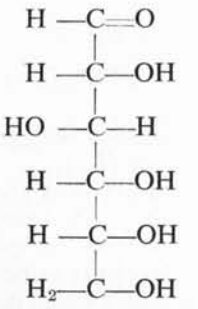
We are working at this Station extensively on the activity of α-decenylsuccinic acid (XXIII) on water permeation. Kuiper (51) has shown that it encourages entry of water into root hairs. It causes bloating of the germ tubes of both our fungi, *Monilinia* and *Stemphylium*. Is the effect due to excessive entry of water?

Then, too, there are the fat solvents that produce bloating and deformity. These are diphenyl (68), cyclohexene (22) [also acts the same on our fungi], chloronitrobenzenes (IX) (54), 2,6-dichloro-4-nitroaniline (XII) (64) and sulfur (30, 35). These highly fat soluble substances presumably also affect the semipermeable membrane and allow excess water to enter.

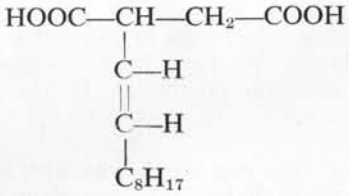
Phenols of various kinds cause cells to bloat, possibly for the same reason as the fat solvents. These are phenol itself (92), hydroquinone (8), thymol (99), methyl-*p*-hydroxybenzoate (92), and α-naphthol (91).



XXI. L-Sorbose



XXII. D-Glucose



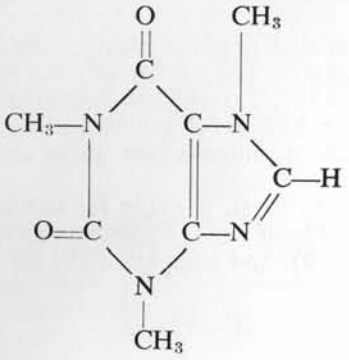
XXIII. α-Decenylsuccinic acid

Altered mitosis. It seems that if the nuclei are inhibited from dividing, the cell swells to accommodate the extra nuclear material within a cell. If so, then mitotic poisons are involved in bloating and possibly excessive branching (37). The bloating here is not due to excess water, but to excess nuclear material.

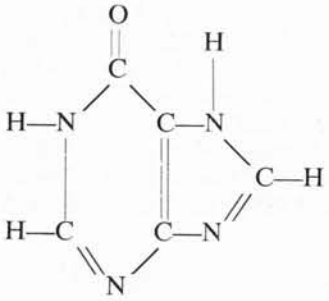
Caffeine (XXIV) is a purine that is said to be mutagenic for a fungus by Fries and Kihlman (24).

Accordingly we tested it and numerous other purines and pyrimidines and we found that many of them produce bloating or excessive branching or both. The effective purines we have found are: caffeine (XXIV) and xanthine, but not hypoxanthine (XXV). The effective pyrimidine diones are thymine, barbituric acid, isobarbituric acid, alloxatin, and actidione (XV). Several 5-membered nitrogen heterocyclic diketones or ketone-thiones that are effective in our tests are phthalimide, hexahydrophthalimide, succinimide, hydantoin, oxazolidine dione, thiazolidine dione, and rhodanine.

From the literature we find some other compounds that appear to



XXIV. Caffeine



XXV. Hypoxanthine

induce giant cells by inhibiting mitosis. These are: actidione (97), camphor (4), and borneol (87).

Spoerl and Pullman (83) report the bloating action of several purine derivatives such as ribosenucleic acid (we found deoxyribose nucleic acid is effective), adenine, 6-mercaptopurine (XXVI), kinetin (XXVII), and α-hydroxy-2-mercaptopurine.

There is surely much more to learn here.

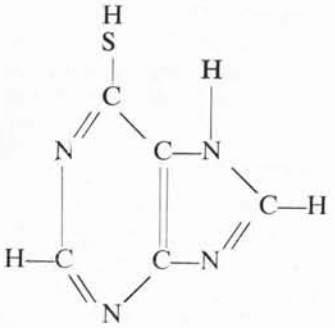
Reducing the Sporulation Response

A striking morphological change in fungi that is affected selectively by fungicides is sporulation. We have been concerned with this problem for many years as a practical possibility. It has theoretical interest to problems of differentiation.

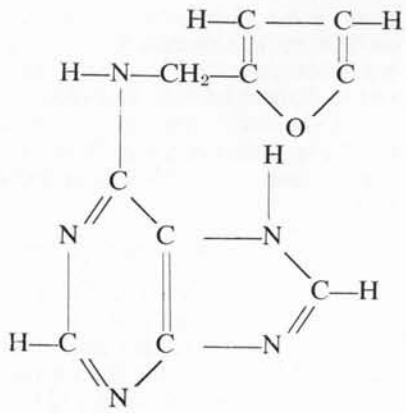
Effect of metal robbing. Our first work was with chelators. Bortels (7) showed nearly 40 years ago that metals, especially copper, are necessary for spore formation in *Aspergillus niger*. If so, then one would selectively

reduce sporulation by robbing the fungus of metal. Our first report (73) showed that this could be accomplished with dimethylglyoxime (XX), a metal chelator.

The chelator work was followed up later (43). We took a list of random metal chelators from Martell and Calvin's text. Every chelator but one reduced sporulation of *M. fructicola*. The exception (*p*-nitrobenzeneazo-α-naphthol) increased sporulation. A few other chelators, such as copper propionylacetonate and copper *o*-benzoylbenzoate, enhance sporulation at low concentrations (37). Presumably they carry the needed copper into the cell and release it to the fungus for the sporulation process.

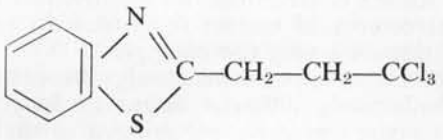


XXVI. 6-Mercaptopurine

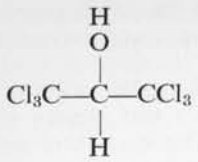


XXVII. Kinetin

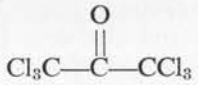
During an intensive screening program to find antisporulants we discovered two potent ones. The first was 2-(trichloropropyl)benzothiazole (XXVIII) (44). It inhibits sporulation of *M. fructicola, S. sarcinaeforme, B. cinerea,* and *A. niger,* but not *Penicillium* sp. Its action can be reversed by copper sulfate and pulegone. The second was hexachloro-2-propanol (XXIX) (45). The 2-keto tautomer (XXX) is not effective. Hexachloro-2-propanol is effective on the organisms just listed including the *Penicillium* sp., but not for *Cladosporium cucumerinum*. It is probable that the compound acts as a reasonably strong acid by virtue of the electron sinks at either end of the molecule. It bleaches the black fungus on which it is effective, *S. sarcinaeforme,* presumably by robbing the phenol oxidase of its copper. It does not bleach the black fungus on which it is not effective, *C. cucumerinum*. Presumably, the color of *C. cucumerinum* is due to some oxidase other than phenol oxidase.



XXVIII. 2-(Trichloropropyl)-benzothiazole



XXIX. Hexachloro-2-propanol



XXX. Hexachloro-2-propanone

Effect of fat solvents. It is interesting that many of the deformity-inducing fat solvents also seem to reduce sporulation. Horsfall and Rich (43) show a list of effective hydrocarbons but an almost equally long list of ineffective fat solvents. In the literature we find evidence that the following are listed as effective antisporulants: benzene (36), chloronitrobenzenes (81), diphenyl (34), and dibromotetrachloroethane (17).

Other miscellaneous compounds from the literature that are said to reduce sporulation are dichloroisocyanuric acid (17), ethionine and related compounds (5), thiosemicarbazide (25), and actidione semicarbazone (33).

Enhancing the Sporulation Response

In an extensive screening test, one also finds compounds that enhance sporulation. Horsfall and Rich (43) have shown that although ketones in general seem to reduce sporulation of *M. fructicola* in culture, α-β-unsaturated ketones enhance sporulation of *M. fructicola.*

Gassner and Nieman (27) report that silver nitrate, lead nitrate, and ceresan promote sporulation of two species of *Tilletia* in soil suspension cultures.

At any rate there is a rich field here for many Ph.D. theses on the biochemistry of differentiation and its alteration with synthetic chemicals. In fact, Reid (70) has used this approach to contrast the respiratory patterns of conidial formation and hyphal growth of *Fusarium.* He contrasted glucose with acetate as substrates for growth and sporulation and said that his "results could be interpreted to mean that energy for growth is obtained from glycolysis and fermentation when glucose is the substrate, while energy for sporulation is obtained through the citric acid cycle."

He instances semicarbazide, an aldehyde trap, that inhibits growth and sporulation. We reported on the fungitoxicity of aldehyde traps several years ago (46). Reid's paper stimulated us to examine our data for aldehyde traps as antisporulants. Several hydrazines and semicarbazides do, in fact, reduce sporulation of *M. fructicola.*

SELECTIVITY BETWEEN ANALOGUE AND ANALOGUE

Aside from studies of structure and effectiveness against fungi, slight changes in the structures of certain fungicides do change chemical responses. We will discuss a very few examples.

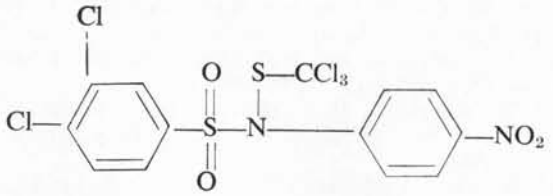
Among dithiocarbamates, exhaustively presented by Thorne and Ludwig (87), distinctively different means of fungitoxicity are shown between the two main types. By replacing a methyl group of sodium dimethyldithiocarbamate (NaDDC) by a hydrogen atom, the chemistry of the resulting vapam is drastically different from NaDDC. Dialkyl DDC,

quite stable above pH 5.5, is held to be toxic in the DDC-form presumably involving copper. Vapam, nabam, etc., vary in stability and readily decompose. Isothiocyanate, one decomposition product, is held to be a possible toxicant that attacks cellular thiols.

Subtle changes in the cyclohexene ring of captan drastically alters selectivity in control of powdery mildew diseases. If four hydrogen atoms are removed from the captan ring (XIX) to form phaltan powdery mildew can be controlled (66). Captan is ineffective against these diseases. Effectiveness of phaltan may lie in its ability to produce toxic vapors. Our colleague Dr. S. Rich (Unpublished) has shown that fungitoxic vapors arise from dry deposits of phaltan on rose leaves while little or none arise from similar deposits of captan.

Fungitoxicity to germinating spores is very pronounced when a trichloromethylthio group is attached to many organic compounds. However, this is not universal. We (54) compared the chemistry of several R-S-CCl_3 compounds showing poor fungitoxicity with that of captan (XIX). Those R-S-CCl_3 compounds (R=imide or sulfone), which are broken down at the R-S bond by thiols giving R-H, thiophosgene, and disulfide, are highly fungitoxic. Carbazole-S-CCl_3, which is not decomposed by thiols at the R-S bond, is weakly fungitoxic. Infrared absorption analyses suggest that thiols replace a chlorine atom of carbazole-S-CCl_3 forming addition products. Steric hindrance of carbazole probably protects the R-S bond of this compound.

N-Trichloromethylsulfenyl-3,4-dichlorobenzenesulfone-4-nitroanilide (XXXI) presents an unusual case. This compound is quickly attacked at the R-S bond by thiols to give R-H, thiophosgene and disulfide like captan and sulfone-S-CCl_3, but it is less toxic than the sulfonamide, alone, to spore germination of *Stemphylium sarcinaeforme* and *Monilinia fructicola.* Also, it fails to evolve CS_2 from treatments of spores. (CS_2 is a decomposition product of thiophosgene). Recent work suggests that when cellular thiols attack sulfonamide-S-CCl_3, the decomposition products react together causing self-detoxication.



XXXI. N-Trichloromethylsulfenyl-3,4-dichlorobenzenesulfone-4-nitroanilide

Excess thiols detoxify captan and sulfone-S-CCl_3 but do not reverse fungitoxicity. This would suggest that toxicity is due to decomposition products and not oxidation of cellular thiols. However, with the sulfonamide analogue, fungitoxicity is reversed as well as detoxified by excess thiols. Presumably, toxicity in this R-S-CCl_3 analogue is due to oxidation of thiols. Toxicity of carbazole-S-CCl_3 can be reversed by washing cells or by adding excess thiols. Presumably, toxicity occurs by forming weakly bound addition-products with cellular thiols.

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