# PARTIAL CHEMOTHERAPY OF THREE CEREAL VIRUSES AND TOBACCO MOSAIC VIRUS WITH CERTAIN ANALOGUES OF PURINE AND PYRIMIDINE AND SEVERAL OTHER ORGANIC COMPOUNDS

by

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## INTRODUCTION

Recent progress in the study of virus chemothreapy has raised the hope of controlling virus diseases of plants through this means. Appearing in the literature have been some experimental evidence that the development of viruses in growing plants can be completely inhibited by the use of chemicals at the proper time (8, 22). Possible cases of chemical curing of plants or plant parts from virus infection have also been reported (9, 29, 33, 35, 39).

Because of localized distribution and, perhaps, availability of no efficient methods of quantative assay, cereal viruses have not received any attention from investigators working on plant virus chemotherapy, even though the prevalence of virus diseases in certain cereal growing areas calls for such attention. With a rather short growing period cereals if once cured from virus infection are not apt to be reinfected. It is perhaps with crops of this type that chemical control might be first successfully applied. Even a considerable delay of disease development in such a crop would also mean a great reduction of losses.

The present study has been carried out with a primary interest in seeking for compounds that might be inhibitory against cereal viruses. Three types of compounds, namely, plant growth regulators, antibiotics, and analogues of purine and pyrimidine have been tested for their effects on four different viruses including three manually transmissible cereal viruses and one sweet potato strain of tobacco mosaic virus. The last named virus was used for comparison as considerable work has already been done with it.

## REVIEW OF LITERATURE

Stoddard (39) working with the X-disease of peach reported curing of

infected buds by treatments with zinc sulfate, calcium chloride and sulfa drugs. This was the first fruitful attempt of plant virus control through chemotherapy. Since then there have appeared in the literature many reports on this line of study, and a great number of compounds have been tested for their possible activities against plant viruses. Of those compounds substantially inhibiting virus multiplication or annulling the effect of virus in plants, analogues of purine and pyrimidine, antibiotics and plant growth regulators have been most widely studied. The following is a brief review of the use of compounds of these three types as well as others. An excellent review of all literature up to 1955 on the chemotherapy of viruses has been given by Matthews and Smith (27).

## Analogues of Purine and Pyrimidine

Noting that substituted purines had an effect on the metabolism of Tetrahymena (a protozoan) and on the development of malignant cells in mice, Matthews introduced purine analogues into the study of chemotherapy of plant viruses (20, 22). He found that 8-azaguanine (guanazolo), the triazolo analogue of guanine, when sprayed as a 0.1 percent suspension in water or a solution in 0.1 percent $NaHCO_3$ on leaves caused marked reduction of local lesions and a delay or complete inhibition of systemic development of lucern mosaic virus in Nicotiana glutinosa and N. tabacum. The compound was also effective in delaying the development of systemic infection with cucumber mosaic virus in cucumber if the virus was introduced by mechanical means. Because of the fact that incubating lucern mosaic virus in expressed sap with 8-azaguanine did not affect the infectivity materially, the inhibitory effect of this compound was thought to occur within the host cells. This effect was annulled by the presence of certain natural purines such as adenine, guanine,

and probably hypoxanthine (Matthews, 20).

Treatment of tobacco plants with 8-azaguanine caused a few days' delay in the appearance of systemic symptoms of tobacco mosaic virus infection, but, never prevented eventual development of the virus (Kutsky, 13). With turnip yellow mosaic virus, the compound had no effect if fairly large Chinese cabbage plants were used; however, with small plants treatment with 8-azaguanine delayed the development of symptoms as with tobacco mosaic virus (Matthews, 24). In comparisons made with leaf discs the compound was found to be less effective against tobacco mosaic virus than certain 2-thiopyrimidines (Mercer, et al., 28). With spotted wilt virus in tomato and N. glutinosa, potato virus X and Y in potato and tobacco, and pea mosaic virus in pea, 8-azaguanine had only slight or negligible effects (Matthews, 22).

8-Azaguanine treatment has not been reported to prevent further systemic spread and development of virus once systemic movement has begun (Matthews, 22). One case, however, was noted by Millikan and Guengerich (29) in which 8-azaguanine treatments freed two plum buds from infection of necrotic ring spot virus among a total of 60 buds variably treated.

The mechanism of virus inhibitory action of 8-azaguanine has been studied by Matthews (23). Through nucleic acid analysis he was able to detect a deficiency of about 3.5 percent in guanine in the ribonucleic acid taken from tobacco mosaic virus from the azaguanine treated plants. This deficiency was expected to be due to replacement of some guanine by 8-azaguanine. Isolation of 8-azaguanylic acid from hydrolysates of virus nucleic acid gave confirmation to this expectation. Virus incorporated with 8-azaguanine was less infectious than the control population as revealed by a lower ratio of infectivity to total virus from treated plants. With turnip yellow mosaic virus similar decreases in infectivity of virus preparations containing 8-azaguanine were observed.

The inhibitory activity of 2-thiouracil, an analogue of pyrimidine, against plant viruses was first reported by Commoner and Mercer (2). When tested on tobacco mosaic virus in isolated leaf discs of tobacco floated on culture solution, the compound inhibited virus synthesis at rather low concentrations. Complete inhibition was obtained at a concentration of $4.3 \times 10^{-5}$ M. The inhibitory activity of the compound was partially reversed by the simultaneous presence of an excess of uracil (Commoner and Mercer, 3). Nichols (31) studying the effect of environmental factors on virus inhibitory activity of 8-thiouracil found that the compound was more effective in reducing virus amount in the presence of light than in the dark. Bawden and Kassanis (1) reported that thiouracil inhibited tobacco mosaic virus production most when conditions were such that without it the most virus would be produced. The rate of virus production was decreased by spraying the compounds on plant leaves, but, was more markedly affected by floating leaves in a solution containing it. In addition, they found that 2-thiouracil reduced the multiplication of potato virus X and Y and henbane mosaic virus in tobacco leaves floated on solutions containing the compound. Gray (6) working with southern bean mosaic virus in bean and tobacco mosaic virus in tobacco and N. rustica found that 2-thiouracil decreased local lesions and inhibited systemic development of the viruses. Holmes (9) reported that systemic necrosis and death of certain lines of tobacco hypersensitive to tobacco mosaic virus could be prevented by watering the plants with five mg. of thiouracil daily for four to 12 days. 2-Thiouracil was also inhibitory towards the multiplication of a stone fruit virus (PLMV) in cucumber as determined by spectrophotometric and biological means (Kirkpatrick and Linder, 11). The curing of plum buds infected with necrotic ring spot virus by 2-thiouracil treatment also has been reported (Milliken and Guengerich, 29).

With Rothamsted strain of tobacco necrosis virus this compound was effective in tobacco but not in the French bean. With broad bean mosaic, lucern mosaic virus and cucumber mosaic virus 2-thiouracil had little or no effect (Bawden and Kassanis, 1).

Evidence that 2-thiouracil is incorporated into virus nucleic acid was presented by Jeener and Rosseels (10) and Matthews (25). Using $S^{35}$ labeled thiouracil they found activity in the virus ribonucleic acid but not in the virus protein. The amount of thiouracil present in ribonucleic acid was found to be about 20 percent of the virus uracil by Jeener and Rosseels and 3.5 percent by Matthews.

In addition to 2-thiouracil and 8-azaguanine, a number of purine and pyrimidine analogues have been shown to produce inhibitory effects on virus multiplication. Mercer et al. (28) tested seven analogues of pyrimidine and two analogues of purine against tobacco mosaic virus in tobacco leaf discs, and found that 2-thiocytosine, 2-thiothymine, and 2,6-diaminopurine inhibited the multiplication of the virus. The inhibitory action could be annulled by uracil but not by cytosine or thymine. In screening tests which involved using 31 purine analogues and 10 naturally occurring purines, Schneider (38) found that 8-azaadenine, 2,6-diaminopurine, 2-azaadenine, benzimidazole, 6-nitrobenzimidazole, 2-ethyl-5-methylbenzimidazole, thioguanine, 2-methylthioadenine and 2-iodoadenine were also effective in decreasing order against tobacco mosaic virus. Kurtzman et al. (12) reported the inhibition of tobacco mosaic virus multiplication in tissue cultures by 6-methylpurine and 6-chloropurine. 2,4-Dithiouracil has a delaying effect on tobacco mosaic virus production when sprayed on tobacco plants at concentrations around 0.01 M in solution in warm water, However, the effect is much less than that produced by 2-thiouracil (Matthews and Smith, 27).

Analogues of purine and pyrimidine with no inhibitory effect on plant viruses have been mentioned by Matthews and Smith (27).

## Antibiotics

Comparatively less work has been done with antibiotics as plant virus chemotherapeutants. Manil, whose work has been briefly cited by Matthews and Smith (27), tested the inhibitory effect of antibiotics on tobacco mosaic virus and found the ineffectiveness of penicillin, streptomycin, tyrothricin and actinomycetin. Leben and Fulton (17) using an agar plate method in which inoculated half-leaves were placed on agar media containing the test compounds and incubated in the dark found that streptothricin and terramycin inhibited the development of local lesions by tobacco necrosis virus and tobacco ring-spot virus in cowpea leaves. Terramycin also showed some activity against tobacco mosaic virus in detached tobacco leaves. As cited by Matthews and Smith (27), Beale and Jones testing a number of antibiotics against tobacco mosaic virus and potato yellow dwarf virus reported that terramycin, aureomycin, chloromycetin, penicillin and streptomycin did not affect virus multiplication. Kutsky (13) with tobacco stem tissue cultured on agar medium found that terramycin, streptomycin and subtilin had no effect on the amount of tobacco mosaic virus produced. Kirkpatrick and Linder (11) applying compounds to whole plants through vacuum infiltration found that chloramphenicol (chloromycetin) reduced the multiplication of tobacco mosaic virus in tomato plants and a stone fruit virus (PLMV) in cucumbers.

Schlegel and Rawlins (37) tested the effect of an antibiotic from an Actinomycete, classified as Nocardia formica, on tobacco mosaic virus multiplication in leaf-discs floated on solutions containing the antibiotic. They observed 69-90 percent inhibition of virus multiplication. Since the inhibition

effect of this antibiotic is essentially independent of light conditions it was thought that the antibiotic may act primarily on virus multiplication rather than on host metabolism. The same antibiotic was later studied on intact plants by Gray (6). He found the antibiotic sprayed on leaves markedly inhibited both the production of local lesions and systemic infections by southern bean mosaic and tobacco mosaic viruses in bean and tobacco plants. The antiviral activity of this antibiotic was similar to, but not as lasting as, that of thiouracil on the viruses in beans and tobacco plants. Applied to roots of Pinto bean plants, the antibiotic also reduced the number of local lesions in primary leaves. Treatment of the viruses in vitro with partially purified and pure preparations of the antibiotic did not reduce the activity of either virus.

Gray (8) with another antibiotic, Cytovirin, isolated from the culture filtrate of a Streptomyces species, found that spraying of the antibiotic at concentrations as low as one ppm of the pure preparation completely prevented lesion formation by southern bean mosaic virus in bean, and a spray level of 0.5 ppm completely prevented local lesion production by tobacco mosaic virus in N. rustica. When sprayed at 100 ppm of crude preparation to leaves the antibiotic was also effective in preventing systemic infections by tomato spotted wilt virus in tomato plants and tobacco mosaic virus in tobacco plants. However, the practical use of this antibiotic as a chemotherapeutant is limited by its toxic effects on both animals and growing plants.[1]

## Plant Growth Regulators

Locke (18) treated leaf-roll infected potatoes with 0.2 percent 2,4-dichlorophenoxyacetic acid and reported the complete masking of symptoms in the

1 Personal letter to Dr. W. H. Sill, Jr. by Reed A.Gray, Merck Sharp and Dohme Res. Laboratories; Rahway, N. J.

new growth produced after treatment and in the first vegetative generation of plants grown from the tubers of treated plants. Kutsky and Rawlins (14) using tissue culture technique in which cross sections of the upper internodes of young mosaic infected tobacco stems were placed on an agar medium in vials found that naphthalene acetic acid at 1.0 mg./l. caused significant reduction of tobacco mosaic virus. This concentration, however, is very close to the toxic concentration of this compound for cultured tobacco tissue. Lower concentrations had no effect. Kutsky (13) used the same technique as Kutsky and Rawlins and found that indolebutyric acid at 10 and 100 mg./l. was effective in reducing the virus concentration of the tissue culture. Phenylacetic acid, phenylpropionic acid and phenylvaleric acid were found ineffective at concentrations that the plant tissues could tolerate. Nichols (30) applied naphthaleneacetic acid and indolebutyric acid to tobacco plants as a spray at 100 mg./l. and found that the treatments retarded development of symptoms and decreased severity of tobacco mosaic symptoms. No concentration of these substances was found to prevent all mosaic symptoms without plant damage. Davis (4) treated N. glutinosa with 4-chloro-3,5-dimethylphenoxyethanol through soil application and found that the treated plants when inoculated with tobacco mosaic virus produced only about 1/3 as many local lesions as did untreated plants. Maramorosch (19) reported that gibberellic acid at 100 ppm reversed stunting caused by corn stunt virus in corn, by aster yellows virus in Chinese aster and by wound tumor virus in crimson clover. Although stunting could be overcome to a considerable degree by the acid, diseased plants retained other signs of virus infection. Leafhopper vectors were able to recover the respective viruses from plants treated with gibberellic acid as readily as from untreated controls.

Miscellaneous Compounds

In addition to the above mentioned groups of compounds, certain dyestuffs, metal ions, carboxylic acids, amino acids and others have also been reported to possess some inhibitory effect on plant viruses.

Takahashi (40) using a detached leaf technique and assaying virus by inoculation to N. glutinosa found that malachite green decreased the amount of tobacco mosaic virus in tobacco. The growth of virus tumors from Rumex acetosa L. was found by Nickell (32) to be inhibited by malachite green, methylene blue and crystal violet at concentrations above 0.1 ppm. Norris (33) cultured young shoots from virus X infected potato tubers in flasks containing liquid nutrient plus malachite green (3 ppm), and reported that, of 16 shoots which survived the treatment, one was proved to be free from the virus. The treatment also reduced the virus content of other shoots to a very low level.

Of metal ions reported to affect virus multiplication the zinc ion has received the most attention. Stoddard (39) stated that zinc sulfate was effective as a chemotherapeutic agent against the X-disease of peach. Rumley and Thomas (35) treated carnation cuttings with zinc sulfate and calcium chloride, using 12 cuttings for each treatment. About half of the resultant plants were found to be free of virus. Weintraub et al. (41) floated tobacco mosaic virus infected N. glutinosa leaves on solution containing zinc sulfate or zinc chloride and found marked reduction in the number of local lesions produced by tobacco mosaic virus in bean leaves which were dipped in zinc sulfate or calcium chloride for 10 minutes at 10 minutes after inoculation.

Matthews and Proctor (26) in an attempt to explain the daily variation in susceptibility of plants to viruses found a striking increase of citric acid and succinic acid content in plants that became resistant after being

grown under continuous artificial illumination. By spraying a number of organic acids on plants, they found that certain di- and tricarboxylic acids reduced the number of local lesions caused by tobacco necrosis virus in bean. Citric and succinic acids were particularly effective without causing plant damage. These acids were thought to interfere with the process of virus establishment rather than multiplication. Their effect can be partially or completely annulled by spraying the plants with solutions of certain metal ions such as caesium, calcium, magnesium, barium and aluminum. Matthews and Proctor speculated that metal ions may play some part in the process of infection by a tobacco necrosis virus whereas the organic acids may act by sequestering metal ions in the leaf.

The effect of some amino acids and related compounds on plant viruses has been studied by Ryjkoff as reviewed by Matthews and Smith (27), Schlegel and Rawlins (36) and Matthews and Proctor (26). When tested against tobacco mosaic virus, glutamic acid, threonine, lysine and cysteine were powerful inhibitors of the necrotic reaction in N. glutinosa and of virus multiplication in tobacco according to Ryjkoff, and l-isoleucine and ethionine were inhibitory to virus multiplication in tobacco leaf discs according to Schlegel and Rawlins. Matthews and Proctor (26) listed 10 amino acid analogues including ethionine as ineffective against the development of tobacco mosaic virus in tobacco, cucumber mosaic virus in cucumber and lucern mosaic virus in tobacco when sprayed on leaves at about 0.01 M.

Weintraub and Kemp (42) tested a number of heterocyclic and other organic compounds on tobacco mosaic virus in N. glutinosa, using a half-leaf technique. The following compounds were found effective in varying degrees: 2-thiophene-carboxaldehyde thiosemicarbazone, 2-benzoyl thiophene, 2-acetyl thiophene,

2-furaldehyde, 2-furaldoxime, 2-nitrofurane, furamide, acetophenone, veratraldehyde and propiophenone. The main effects of these compounds were reduction in the total number of lesions, delay in symptom expression and decrease in virus multiplication as indicated by small lesions on the treated half-leaves. Porter et al. (34) using a stem inoculation technique found that two substituted carbamates, furfuryl carbanilate and furfuryl 5-chloro-2-methylcarbanilate, suppressed symptom formation in Pinto bean stems by southern bean mosaic virus. Application of the compounds in lanolin immediately following stem inoculation resulted in almost complete suppression of symptoms without apparent injury to plants. Furfuryl 5-chloro-2-methylcarbanilate when used in combination with the so-called "2-phase" carrier caused symptom suppression accompanied by localized injury of the stem.

## MATERIALS AND METHODS

### Compounds Used

The following is a list of test compounds used which included (1) purine and pyrimidine analogues, (2) plant growth regulators and (3) several antibiotics known as Acti-diones. The name "Acti-diones" is commercially given to preparations with cycloheximides as the active ingredient. When used, all these compounds were brought into solution in distilled water unless otherwise indicated.

(1) Analogues of purine and pyrimidine and related compounds.

8-azaadenine

8-aza-2,6-diaminopurine

8-azaguanine (used as a solution in 0.1 percent sodium bicarbonate)

8-azahypoxanthine

8-azaxanthine

6-chloropurine

8-chloroxanthine

2,6-diaminopurine sulfate·$\frac{1}{2}$ $H_2O$ (used as an aqueous solution if the concentration needed was 300 mg./l. or lower; used as a solution in 0.1 percent $NaHCO_3$ if higher concentrations were needed)

6-methylpurine

6-oxy-2,6-dithiopurine

2-thioadenine

6-amino-4-oxy-2-thiopyrimidine

2-amino-4-oxy-6-thiopyrimidine

5-bromouracil

4,5-diamino-6-oxy-2-thiopyrimidine

diazouracil

2,4-dithiopyrimidine

5-methyl-4-oxy-2-thiopyrimidine

6-methyl-4-oxy-2-thiopyrimidine

5-nitrouracil

2-thiocytosine

2-thiouracil

benzimidazole

6-nitrobenzimidazole

(2) Plant growth regulators.

2,4-dichlorophenoxyacetic acid

naphthaleneacetic acid

indolebutyric acid

(3) Acti-diones.

Actidione

Acti-dione M

Acti-dione S

Acti-dione T

## Viruses and Plants

Viruses on which the effect of these compounds has been tested were three manually transmissible cereal viruses, e.g. wheat streak mosaic virus, barley stripe mosaic virus and brome grass mosaic virus, and a sweet potato strain of tobacco mosaic virus. The cereal viruses have been kept in pure cultures for several years in the wheat mosaic laboratory at the Department of Botany and Plant Pathology, Kansas State College, and studied by Lal and Sill (16) and Lal (15) for their synergistic effect in wheat. The sweet potato strain of tobacco mosaic virus was received from Dr. O. H. Elmer of the same department who originally isolated it from a diseased sweet potato plant. This virus was first regarded as sweet potato mosaic virus (Elmer, 5) and later proved to be a strain of tobacco mosaic virus. It differs from the ordinary strain by producing a systemic disease in sweet potato and causing local lesions in egg plants (Dr. O. H. Elmer, personal communication).

As a systemic host of the cereal viruses, the wheat variety Marquillo-Oro-Pawnee selection no. 462666 was used throughout the experiment. Datura stramonium L. was the other host of the brome grass mosaic virus employed. When inoculated the plant produced etched rings or lesions about 1-2 mm. in diameter, with normal green or light green islands at the center (PLATE I). The local lesion response of this plant to this virus was found in the course

EXPLANATION OF PLATE I

A leaf of Datura stramonium L. showing etched rings or faint chlorotic lesions on the right half as a result of the inoculation with brome grass mosaic virus. The left half served as the control.

PLATE I

of the study. Nicotiana tabacum var. Havana 38 and N. glutinosa were used as hosts of the strain of tobacco mosaic virus and gave systemic symptoms and local lesions respectively.

All test plants were grown under greenhouse conditions with the temperature adjusted around 75°F. in winter seasons and fluctuating between 50-102°F. in the summer seasons. Most of the experiments were carried out in winter when the temperature remained fairly constant. For growing wheat plants 5-inch pots were used. Usually 10 plants were grown per pot. Datura stramonium, N. glutinosa, and N. tabacum plants were grown singly in pots with upper diameters of five, four and three inches respectively. Weekly fumigation of the greenhouse using Plantfume 103 (a commercial smoke generator containing 15 percent tetraethyl dithiopyrophosphate as the active ingredient) was adopted to minimize virus spread by possible vectors.

## Inoculation of the Viruses

For preparing inocula of cereal viruses fresh leaf tissues of infected wheat plants were ground by mortar and pestle, the sap was forced through several layers of cheesecloth and diluted differently for different purposes. If used for the inoculation of wheat plants, dilution was made according to a proportion of 10 ml. water per gram of leaf tissue; if used for the inoculation of Datura stramonium plants, the sap was diluted with 0.1 percent $K_2HPO_4$ to 1:100, a concentration that gave a satisfactory number of local lesions. Inocula of tobacco mosaic virus were prepared by diluting a partially purified virus preparation of unknown concentration to 1:32 or 1:160 depending on the experiment.

Wheat plants at the 4-leaf stage were inoculated by means of a piece of

cheesecloth folded into several layers and moistened with inoculum containing carborundum. Inoculation was made effective by rubbing the leaves with the cheesecloth held between thumb and pointing finger. Four strokes were generally employed. For the inoculation of Datura stramonium, N. glutinosa, and tobacco plants, inoculum was rubbed over the leaf surface, which had been evenly dusted with carborundum, using a cotton swab. Caution was taken to avoid damage of the leaf tissue. No washing with water followed the inoculation.

### Application of Test Compounds

In most of the experiments the compounds were applied to the leaves by means of a small hand atomizer. Usually two to four applications were made. In other experiments which involved testing 8-azaguanine on the three cereal viruses and 2-thiouracil and three plant growth regulators on wheat streak mosaic virus, the compounds were given to the plants through the roots as soil drenches. The method of testing compounds against tobacco mosaic virus in detached leaves will be given separately.

### Measuring Effects of Test Compounds

The inhibitory effect of compounds against the viruses in hosts infected systemically was measured by recording the mean time taken for the control and treated plants to show systemic symptoms, and that against viruses in their local lesion hosts was measured by comparing local lesion numbers. The comparative severity of the virus reaction in systemic symptom hosts was also observed.

## EXPERIMENTAL RESULTS

### With Purine and Pyrimidine Analogues and Related Compounds

Tests on Cereal Viruses. Preliminary Test on Wheat Streak Mosaic Virus and Barley Stripe Mosaic Virus. Table 1 summarizes the results of the preliminary test on the effect of 21 compounds on both wheat streak mosaic virus and barley stripe mosaic virus. Test compounds, except 8-azaguanine which was dissolved in 0.1 percent $NaHCO_3$, were used as aqueous solutions at three concentrations. With those compounds having a solubility in water lower than 500 mg./l., the highest concentration used approximated the saturation point of the compounds in cold water. All test compounds at concentrations indicated were not injurious to wheat growth although slight amounts of leaf-tip burning were observed on plants treated with 2-thiouracil at 40 mg./l. When tested on wheat streak mosaic virus, 8-azaguanine, 2,6-diaminopurine, 2-thiouracil, benzimidazole, 6-nitrobenzimidazole, 8-azahypoxanthine and 6-oxy-2,8-dithiopurine seemed to cause a slight delay in symptom expression in treated plants. None of the compounds tested was effective against barley stripe mosaic virus.

Further Test on Wheat Streak Mosaic Virus. Nine different compounds that either showed promise in the preliminary test or were known to be virus inhibitory to certain viruses were further tested on wheat streak mosaic virus. The results of this series of experiments are summarized in Table 2. In this table it may be seen that all test compounds, with the exception of 6-oxy-2,8-dithiopurine, caused some retardation in symptom expression. The slight increase in effect with an increase in concentration was observed for 8-azaguanine, 2,6-diaminopurine, 2-thiouracil, benzimidazole, 6-nitrobenzimidazole and 6-chloropurine. With 2-thiouracil this tendency might, however, be due in part to phytotoxicity. The compound at concentrations of 40 mg. and 80

Table 1. Results of preliminary tests on the inhibitory effect of substituted purines, pyrimidines and structurally related compounds on wheat streak mosaic virus (WSMV) and barley stripe mosaic virus (BSMV) in wheat.*

| Test Compounds | Treatment Concentrations mg./l. | Test on WSMV: No. days taken to show symptoms, mean of 20 plants | Test on WSMV: Incubation period lengthened in percentage | Test on BSMV: No. days taken to show symptoms, mean of 25 plants | Test on BSMV: Incubation period lengthened in percentage |
|---|---|---|---|---|---|
| Control | | 6.05 | | 7.56 | |
| 8-Azaadenine | 2.5 | 6.40 | 0.35 | 7.32 | -0.24 |
| | 5 | 6.40 | 0.35 | 7.24 | -0.32 |
| | 10 | 6.30 | 0.25 | 7.52 | -0.04 |
| 8-Aza-2,6-diaminopurine sat. soln. | (5) | 6.25 | 0.20 | 7.50 | -0.06 |
| 8-Azaguanine | 10 | 6.25 | 0.20 | 7.64 | 0.08 |
| | 20 | 7.10 | 1.05 | 7.96 | 0.40 |
| | 40 | 6.65 | 0.60 | 7.68 | 0.12 |
| 8-Azahypoxanthine | 10 | 6.75 | 0.70 | 7.80 | 0.24 |
| | 30 | 6.95 | 0.90 | 7.32 | -0.24 |
| | 50 | 6.55 | 0.50 | 7.32 | -0.24 |
| 8-Azaxanthine | 10 | 6.35 | 0.30 | 7.58 | 0.02 |
| | 30 | 6.15 | 0.10 | 7.68 | 0.12 |
| | 50 | 6.15 | 0.10 | 7.64 | 0.08 |
| 8-Chloroxanthine | 5 | 6.20 | 0.15 | 7.68 | 0.12 |
| | 10 | 6.10 | 0.05 | 7.84 | 0.28 |
| | 15 | 6.16 | 0.11 | 7.56 | 0.00 |
| 2,6-Diaminopurine sulfate.$\frac{1}{2}$ $H_2O$ | 10 | 6.95 | 0.90 | 7.64 | 0.08 |
| | 20 | 6.60 | 0.55 | 7.12 | -0.44 |
| | 30 | 7.68 | 1.63 | 8.04 | 0.48 |
| 6-Oxy-2,8-dithiopurine | 10 | 6.45 | 0.40 | 7.52 | -0.04 |
| | 20 | 6.55 | 0.50 | 7.60 | 0.04 |
| | 30 | 6.55 | 0.50 | 7.60 | 0.04 |
| 6-Amino-4-oxy-2-thiopyrimidine | 5 | 6.40 | 0.35 | 7.36 | -0.20 |
| | 10 | 6.45 | 0.40 | 7.52 | -0.04 |
| | 20 | 6.10 | 0.05 | 7.60 | 0.04 |

Table 1. (Con't.)

| Test Compounds | Treatment Concentrations: mg./l. | Test on WSMV: No. days taken to show symptoms, mean of 20 plants | Test on WSMV: Incubation period lengthened in percentage | Test on BSMV: No. days taken to show symptoms, mean of 25 plants | Test on BSMV: Incubation period lengthened in percentage |
|---|---|---|---|---|---|
| 2-Amino-4-oxy-6-thiopyrimidine | 5 | 6.20 | 0.15 | 7.60 | 0.04 |
| | 10 | 6.05 | 0.00 | 7.48 | -0.08 |
| | 20 | 6.25 | 0.20 | 7.76 | 0.20 |
| 5-Bromouracil | 10 | 6.00 | -0.05 | 7.40 | -0.16 |
| | 30 | 6.00 | -0.05 | 7.48 | -0.08 |
| | 50 | 6.25 | 0.20 | 7.32 | -0.24 |
| 4,5-Diamino-6-oxy-2-thiopyrimidine | 5 | 6.05 | 0.00 | 7.20 | -0.36 |
| | 10 | 6.42 | 0.37 | 7.68 | 0.12 |
| | 15 | 6.00 | -0.05 | 7.76 | 0.20 |
| Diazouracil | 10 | 6.42 | 0.37 | 7.60 | 0.04 |
| | 20 | 6.40 | 0.35 | 7.48 | -0.08 |
| | 30 | 6.30 | 0.25 | 7.56 | 0.00 |
| 2,4-Dithiopyrimidine | 5 | 6.05 | 0.00 | 7.24 | -0.32 |
| | 10 | 6.15 | 0.10 | 7.36 | -0.20 |
| | 15 | 6.16 | 0.11 | 7.32 | -0.24 |
| 5-Methyl-4-oxy-2-thiopyrimidine | 10 | 6.24 | 0.19 | 7.20 | -0.36 |
| | 30 | 6.15 | 0.10 | 7.24 | -0.32 |
| | 50 | 6.00 | -0.05 | 7.48 | -0.08 |
| 6-Methyl-4-oxy-2-thiopyrimidine | 10 | 6.00 | -0.05 | 7.36 | -0.20 |
| | 20 | 6.05 | 0.00 | 7.40 | -0.16 |
| | 30 | 6.25 | 0.20 | 7.36 | -0.20 |
| 5-Nitrouracil | 10 | 6.20 | 0.15 | 7.24 | -0.32 |
| | 50 | 6.25 | 0.20 | 7.40 | -0.16 |
| | 100 | 6.25 | 0.20 | 7.36 | -0.20 |
| 2-Thiocytosine | 10 | 6.35 | 0.30 | 7.40 | -0.16 |
| | 30 | 6.45 | 0.40 | 7.84 | 0.28 |
| | 50 | 6.55 | 0.50 | 7.64 | 0.08 |
| 2-Thiouracil | 10 | 6.60 | 0.55 | 7.68 | 0.12 |
| | 20 | 6.85 | 0.80 | 7.40 | -0.16 |
| | 40 | 6.60 | 0.55 | 7.76 | 0.20 |

Table 1. (Concl.)

| Test Compounds | Treatment Concentrations mg./l. | Test on WSMV: No. days taken to show symptoms, mean of 20 plants | Test on WSMV: Incubation period lengthened in percentage | Test on BSMV: No. days taken to show symptoms, mean of 25 plants | Test on BSMV: Incubation period lengthened in percentage |
|---|---|---|---|---|---|
| Benzimidazole | 10 | 6.50 | 0.45 | 7.52 | -0.04 |
| | 30 | 6.95 | 0.90 | 7.49 | -0.07 |
| | 50 | 6.68 | 0.63 | 7.52 | -0.04 |
| 6-Nitrobenzimidazole | 10 | 6.70 | 0.65 | 7.48 | -0.08 |
| | 20 | 6.50 | 0.45 | 7.40 | -0.16 |
| | 30 | 6.90 | 0.85 | 7.58 | 0.02 |

* The test compounds were applied as a spray onto leaves. Two applications on successive days began the next day after inoculation.

Table 2. Effect of some substituted purines, pyrimidines and structurally related compounds on wheat streak mosaic virus in wheat.*

| Test Compounds | Treatment Concentrations mg./l. | Trial | No. days taken to show symptoms, mean of 100 plants | Incubation period lengthened in percentage |
|---|---|---|---|---|
| Control | | I | 7.87 | |
| Control | | II | 7.47 | |
| 8-Azaguanine | 100 | I | 7.90 | 0.38 |
| | 200 | I | 7.99 | 1.52 |
| | 400 | I | 8.09 | 2.82 |
| | 800 | II | 7.77 | 4.02 |
| | 1200 | II | 7.82 | 4.69 |
| | 2000 | II | 8.17 | 9.36 |
| 2,6-Diaminopurine sulfate·½ $H_2O$ | 100 | I | 7.99 | 1.52 |
| | 200 | I | 8.37 | 6.35 |
| | 300 | I | 8.32 | 5.72 |
| | 600 | II | 7.78 | 4.15 |
| | 1000 | II | 8.11 | 8.56 |

Table 2. (Concl.)

| Test Compounds | Treatment Concentrations mg./l. | Trial | No. days taken to show symptoms, mean of 100 plants | Incubation period lengthened in percentage |
|---|---|---|---|---|
| 6-Oxy-2,8-dithiopurine | 100 | I | 8.06 | 2.41 |
| | 200 | I | 8.12 | 3.18 |
| | 300 | I | 7.86 | -0.13 |
| 2-Thiouracil | 100 | I | 8.10 | 2.98 |
| | 200 | I | 8.36 | 6.23 |
| | 400 | I | 8.71 | 8.13 |
| approx. | 800 | II | 8.02 | 5.64 |
| Benzimidazole | 100 | I | 7.92 | 0.63 |
| | 300 | I | 8.20 | 4.19 |
| | 500 | I | 8.23 | 4.57 |
| | 1000 | II | 7.86 | 5.22 |
| | 2000 | II | 7.90 | 5.76 |
| 6-Nitrobenzimidazole | 100 | I | 7.88 | 0.13 |
| | 200 | I | 8.16 | 3.69 |
| | 300 | I | 8.37 | 6.35 |
| | 500 | II | 7.96 | 6.56 |
| 8-Azahypoxanthine | 1000 | II | 7.78 | 4.15 |
| | 1500 | II | 7.68 | 2.81 |
| | 2000 | II | 7.74 | 3.62 |
| 6-Chloropurine | 1000 | II | 7.64 | 2.28 |
| | 2000 | II | 7.91 | 5.89 |
| 2-Thioadenine | half-saturated | II | 7.66 | 2.54 |
| | saturated | II | 7.65 | 2.41 |

* The test compounds were applied onto leaves as a spray. Two daily applications began about two hours after inoculation.

mg./l. caused mottling and wrinkling in young leaves and yellowing and burning in old leaves. Difficulty in diagnosing the symptoms on leaves which were mottled and wrinkled was encountered.

Virus control and treated plants if kept for 10 days or longer after inoculation showed symptoms of the same severity. No final prevention of symptom expression was noted on any treated plants.

In addition to the compounds listed, 6-methylpurine has also been tested. This compound is highly phytotoxic to wheat plants. Plants receiving on successive days two applications of a solution at 300 mg./l. mostly died in one to two weeks after the treatment. A few plants that occasionally recovered from the injury later showed severe virus symptoms.

In other experiments 8-azaguanine and 2-thiouracil were applied at five mg., 10 mg. and 20 mg./l. as soil drenches in five daily applications with a total amount of 250 ml. for each pot of five to six plants. Applications were made either before or after inoculation. No prevention of symptom expression was observed. Plants so treated with thiouracil at the highest concentration were markedly stunted and stimulated to tiller excessively.

Further Tests on Barley Stripe Mosaic Virus. Of nine compounds tested further on barley stripe mosaic virus in wheat none was effective enough to cause remarkable delay in symptom expression in the treated plants (Table 3). Crinkling and mottling of yound leaves and tip-burning of outer old leaves were observed with plants receiving 2-thiouracil at 400 mg. and 800 mg./l. 6-Methylpurine was ineffective at concentrations where plant injury occurred.

Tests on Brome Grass Mosaic Virus. Twenty three different compounds were tested for their effect on brome grass mosaic virus in Datura stramonium; eighteen were further tested against the same virus in wheat (Table 4). Reduction in numbers of local lesions, as counted seven days after inoculation,

Table 3. Effect of some substituted purines, pyrimidines and structurally related compounds of barley stripe mosaic virus in wheat.*

| Test Compounds | Treatment Concentrations mg./l. | Trial | No. days taken to show symptoms, mean of 40 plants | Incubation period lengthened in percentage |
|---|---|---|---|---|
| Control | | I | 5.575 | |
| Control | | II | 7.025 | |
| 8-Azaguanine | 100 | I | 5.850 | 4.93 |
| | 200 | I | 5.725 | 2.69 |
| | 400 | I | 5.750 | 3.14 |
| | 800 | II | 7.200 | 2.49 |
| | 1200 | II | 7.250 | 3.20 |
| | 2000 | II | 7.200 | 2.49 |
| 2,6-Diaminopurine sulfate·½ $H_2O$ | 100 | I | 5.400 | -3.14 |
| | 200 | I | 5.475 | -1.79 |
| | 300 | I | 5.500 | -1.35 |
| | 600 | II | 7.250 | 3.20 |
| | 1000 | II | 7.275 | 3.56 |
| 6-Oxy-2,8-dithiopurine | 100 | I | 5.850 | 4.93 |
| | 200 | I | 5.625 | 0.90 |
| | 300 | I | 5.600 | 0.44 |
| 2-Thiouracil | 100 | I | 5.725 | 2.69 |
| | 200 | I | 5.625 | 1.79 |
| | 400 | I | 5.950 | 6.71 |
| approx. | 800 | II | 7.450 | 6.05 |
| Benzimidazole | 100 | I | 5.800 | 4.04 |
| | 300 | I | 5.700 | 2.24 |
| | 500 | I | 5.650 | 1.35 |
| | 1000 | II | 7.400 | 5.34 |
| | 2000 | II | 7.375 | 4.98 |
| 6-Nitrobenzimidazole | 100 | I | 5.450 | -2.24 |
| | 200 | I | 5.600 | 0.44 |
| | 300 | I | 5.700 | 2.24 |
| approx. | 500 | II | 7.300 | 3.91 |

Table 3. (Concl.)

| Test Compounds | Treatment Concentrations mg./l. | Trial | No. days taken to show symptoms, mean of 40 plants | Incubation period lengthened in percentage |
|---|---|---|---|---|
| 8-Azahypoxanthine | 1000 | II | 7.150 | 1.78 |
| | 1500 | II | 7.275 | 3.56 |
| | 2000 | II | 7.225 | 2.85 |
| 6-Chloropurine | 1000 | II | 7.225 | 2.85 |
| | 2000 | II | 7.175 | 2.14 |
| 2-Thioadenine | half-saturated | II | 7.250 | 3.20 |
| | saturated | II | 7.425 | 5.69 |

* The test compounds were applied onto leaves as a spray. The daily applications began about two hours after inoculation.

Table 4. Effect of substituted purines, pyrimidines and structurally related compounds on brome mosaic virus in wheat and Datura stramonium.*

| Test Compounds | Treatment Concentrations mg./l. | In Datura stramonium: No. of local lesions. Mean of 3 leaves Trial 1 | Trial 2 | In wheat: No. of days taken to show symptoms. Mean of 40 plants | Incubation period lengthened in percent |
|---|---|---|---|---|---|
| Control | | 217 | 45 | 7.050 | |
| 8-Azaguanine | 100 | — | — | 7.075 | 0.35 |
| | 400 | 3 | 2 | 7.650 | 8.51 |
| | 1200 | — | — | 7.675 | 8.87 |
| | 2000 | 0 | 0 | 8.325 | 18.09 |
| 2,6-Diaminopurine sulfate·½ $H_2O$ | 100 | — | — | 7.050 | 0 |
| | 300 | 78 | 39 | — | — |
| | 600 | — | — | 7.075 | 0.35 |
| | 1000 | 68 | 34 | 7.000 | -0.71 |
| 8-Azaadenine | 100 | 73 | 9 | 7.025 | -0.35 |
| 8-Aza-2,6-diaminopurine | sat.soln. | — | 12 | 7.025 | -0.35 |
| 8-Azahypoxanthine | 2000 | 157 | 75 | 7.100 | 0.71 |
| 8-Azaxanthine | 1000 | 136 | 114 | 7.175 | 1.77 |
| 6-Chloropurine | 2000 | 36 | 80 | 7.050 | 0 |

Table 4. (Concl.)

| Test Compounds | Treatment: Concentrations: mg./l. | In Datura stramonium: No. of local lesions. Mean of 3 leaves Trial 1 | Trial 2 | In wheat: No. of days taken to show symptoms. Mean of 40 plants | Incubation period lengthened in percent |
|---|---|---|---|---|---|
| 6-Methylpurine | 50 | 91 | 28 | 7.100 | 0.71 |
| 6-Oxy-2,8-dithiopurine | 300 | 97 | 20 | 7.100 | 0.71 |
| 2-Thioadenine | sat.soln. | 114 | 49 | 7.050 | 0 |
| 6-Amino-4-oxy-2-thiopyrimidine | 100 | 68 | 6 | 7.100 | 0.71 |
| 2-Amino-4-oxy-6-thiopyrimidine | 100 | 114 | 32 | 7.100 | 0.71 |
| 5-Bromouracil | 500 | 120 | 8 | 7.000 | -0.71 |
| 2,4-Dithiopyrimidine | 150 | 170 | 20 | 7.125 | 1.06 |
| 4,5-Diamino-6-oxy-3-thiopyrimidine | 150 | 195 | 47 | ——— | ——— |
| Diazouracil | 300 | 37 | 94 | ——— | ——— |
| 5-Methyl-4-oxy-2-thiopyrimidine | 500 | 222 | 145 | ——— | ——— |
| 6-Methyl-4-oxy-2-thiopyrimidine | 300 | 173 | 20 | 7.025 | -0.35 |
| 5-Nitrouracil | 2000 | 140 | 68 | ——— | ——— |
| 2-Thiocytosine | 1000 | 50 | 35 | 7.050 | 0 |
| 2-Thiouracil | 100 | 270 | 210 | 7.050 | 0 |
| | 400 | ——— | ——— | 7.405 | 5.04 |
| approx. | 800 | ——— | ——— | 7.150 | 1.52 |
| Benzimidazole | 1000 | 131 | 26 | ——— | ——— |
| | 2000 | ——— | ——— | 7.175 | 1.78 |
| 6-Nitrobenzimidazole | 300 | 151 | 56 | 7.200 | 2.13 |

* The test compounds were applied onto leaves as a spray. Two applications were made before and two after inoculation.

was observed with Datura plants sprayed with 8-azaguanine, 8-azaadenine, 2,6-diaminopurine, 6-amino-4-oxy-2-thiopurine, 5-bromouracil, and 2-thiocytosine. 8-Azaguanine also caused a delay in symptom development in wheat. The azaguanine treated wheat plants stood out distinctly because of their milder mottling and deeper color. However, this was true for only about two weeks after inoculation. As the inoculated plants aged and the disease advanced the symptom differences between the treatments became inperceptible.

The effect of 8-azaguanine on brome mosaic virus was influenced by environmental conditions. When the incubation period was shortened due to high temperature, the inhibitory effect of this compound became undetectable. Thus, in infected plants with a mean incubation period of about seven days 8-azaguanine at 1200 and 2000 mg./l. caused nine and 18 percent lengthening respectively; while in plants with a mean incubation period of about four days the compound at the same concentrations produced no differences in the incubation period.

Applied through the soil as a drench 8-azaguanine at concentrations up to 800 mg./l. was ineffective. Six daily 25 ml. applications (one before inoculation and five after inoculation) were made to 5-inch pots containing 10 diseased plants per pot. There was no delay in symptom expression at any time.

As a spray 8-azaguanine at concentrations inhibitory to brome grass mosaic virus had no retarding effect on the growth of wheat plants. Datura stramonium receiving 4-daily applications developed a dull appearance with the treated leaves becoming grayish yellow in color.

Tests on Sweet Potato Strain of Tobacco Mosaic Virus. For testing the inhibitory effect of 23 compounds on a sweet potato strain of tobacco mosaic virus in systemic host plants, tobacco seedlings, about 2-3 inches high, with

five leaves were employed. Only the right half of the third leaf was inoculated. Two daily applications of the compounds as leaf sprays were made before inoculation and two followed inoculation. The results of this experiment are shown in Table 5. Of compounds tested, 2-thiouracil at all concentrations, 8-azaguanine at 800 mg. and 1200 mg./l., 2,6-diaminopurine sulfate at 300 mg. and 600 mg./l., and 6-chloropurine at 2000 mg./l. markedly inhibited disease development in treated plants. Expression of symptoms was also slightly delayed by 8-azaadenine at 100 mg./l., 2-thiocytosine at 1000 mg./l. and benzimidazole at 1000 mg./l. All treated plants produced faint chlorotic spots of different size and sharpness on inoculated leaves. No correlation between the systemic symptoms and the number, size and sharpness of the primary local lesions could be seen. One of four plants treated with 8-azaguanine at 1200 mg./l. did not show systemic symptoms until 22 days after inoculation. In a test to determine the presence or absence of active virus, sap from young leaves, taken 15 days after inoculation, produced no local lesions on N. glutinosa, although the sap from inoculated leaves produced numbers of local lesions not significantly different from the control. This indicated that the virus in treated plants moved more slowly than usual from the point of inoculation. A similar observation has been made by Matthews using several tobacco mosaic virus strains (Matthews, 23).

Although the effect of the above mentioned compounds on tobacco mosaic virus in cultured leaves has been repeatedly reported (2, 3, 22, 32), only 2-thiouracil has been known to suppress symptom development when applied to whole plants (6, 9, 11). 2-Thiocytosine had no detectable effect on the systemic development of tobacco mosaic virus and the slight effect of 2,6-diaminopurine and 8-azaadenine could not be dissociated from plant damage as Matthews and Smith stated (27).

Table 5. Effect of substituted purines, pyrimidines and related compounds on a sweet potato strain of tobacco mosaic virus in Nicotiana tabacum and N. glutinosa.*

| Test Compounds | Treatment Concentrations mg./l. | In N. tabacum Incubation period as expressed in days Mean of 4 plants | In N. glutinosa No. of local lesions Mean of 6 leaves on two plants |
|---|---|---|---|
| Control | | 5.75 | 60.6 |
| 8-Azaguanine | 100 | 6.00 | —— |
| | 400 | 7.00 | 45.8 |
| | 800 | 9.00 | —— |
| | 1200 | 12.00 | —— |
| | 2000 | —— | 38.3 |
| 8-Azaadenine | 100 | 7.50 | 49.5 |
| 8-Aza-2,6-diaminopurine | sat. | 6.00 | 49.0 |
| 8-Azahypoxanthine | 2000 | 6.25 | 53.3 |
| 8-Azaxanthine | 1000 | 6.00 | 63.5 |
| 8-Chloroxanthine | 150 | 6.25 | 53.0** |
| 2,6-Diaminopurine | 100 | 6.50 | —— |
| sulfate·½ $H_2O$ | 300 | 8.50 | 31.1 |
| | 600 | 10.75 | —— |
| 6-Oxy-2,8-dithiopurine | 300 | 6.50 | 26.8 |
| 6-Methylpurine | 50 | ——*** | 41.5 |
| 6-Chloropurine | 2000 | 8.75 | 30.1 |
| 2-Thioadenine | sat. | 6.50 | 40.1 |
| 6-Amino-4-oxy-2-thio-pyrimidine | 200 | 6.50 | 45.6 |
| 2-Amino-4-oxy-2-thio-pyrimidine | 200 | 5.75 | 52.8 |
| 5-Bromouracil | 500 | 5.50 | 34.6 |
| 4,5-Diamino-6-oxy-2-thiopyrimidine | 150 | 5.50 | 57.1 |
| Diazouracil | 300 | 6.50 | 49.3 |
| 2,4-Dithiopyrimidine | 150 | 6.25 | 40.0 |
| 5-Methyl-4-oxy-2-thio-pyrimidine | 500 | 6.00 | 48.3 |
| 6-Methyl-4-oxy-2-thio-pyrimidine | 300 | 7.00 | 46.6 |
| 5-Nitrouracil | 2000 | 6.25 | 30.1 |
| 2-Thiocytosine | 1000 | 7.50 | 48.5 |
| 2-Thiouracil | 50 | 8.75 | 54.1 |
| | 100 | 8.75 | 19.0 |
| | 200 | 9.25 | —— |
| Benzimidazole | 100 | 6.50 | —— |
| | 500 | 6.00 | —— |
| | 1000 | 7.50 | 49.0 |
| 6-Nitrobenzimidazole | 100 | 6.50 | —— |
| | 300 | 6.00 | 24.3 |
| approx. | 500 | 6.75 | —— |

* The test compounds were applied as a spray onto leaves. Two daily applications were made before inoculation and two made after inoculation.

** Mean of 3 leaves.

*** Phytotoxicity occurred.

The effect of these compounds on local lesion formation caused by tobacco mosaic virus on N. glutinosa was studied using growing plants. The results are shown in Tables 5 and 6. Of those compounds inhibiting systemic spread of the virus in N. tabacum, 2-thiouracil, 2,6-diaminopurine, 8-azaadenine, and benzimidazole decreased local lesion numbers on N. glutinosa. 2-Thiouracil also markedly delayed lesion formation and caused a reduction of the size of local lesions. Tests with 8-azaguanine, 6-chloropurine and 2-thiocytosine yielded variable results. In one experiment these compounds decreased the number of local lesions; in the other experiment, no such effect could be observed. However, plants receiving 8-azaguanine applications tended to take longer to produce local lesions which were smaller and less necrotic than the controls.

The effects of the compounds on tobacco mosaic virus was also studied in an experiment in which inoculated leaves of N. glutinosa were detached one hour after inoculation and divided along the midrib into two halves with one half incubated in a solution containing the test compound in petri dishes and the other half in distilled water of 0.1 percent $NaHCO_3$ (control for 8-azaguanine and 8-aza-2,6-diaminopurine). The petri dishes were placed on the top of a bench in the greenhouse. The results of this experiment are shown in Table 7. It was revealed in this experiment that, in addition to some known effective compounds, 8-aza-2,6-diaminopurine and 5-methyl-4-oxy-2-thiopyrimidine at 200 mg./l. markedly decreased the number of local lesions and delayed their formation. No phytotoxic effect was observed at the concentration used. 6-Oxy-2,8-dithiopurine, 5-bromouracil and 5-nitrouracil which were effective in decreasing the number of local lesions when applied to growing plants were ineffective or only slightly effective when tested in detached leaves.

Table 6. Effect of substituted purines, pyrimidines and related compounds on a sweet potato strain of tobacco mosaic virus in Nicotiana glutinosa.*

| Test Compounds | Treatment Concentrations mg./l. | No. of local lesions per leaf** |
|---|---|---|
| Experiment 1 | | |
| Control | | 58.1 |
| 8-Azaguanine | 400 | 59.0 |
| | 1200 | 72.7 |
| | 2000 | 93.8 |
| 2,6-Diaminopurine sulfate·½ $H_2O$ | 300 | 63.4 |
| | 1200 | 41.8 |
| | 2000 | 42.5 |
| 6-Chloropurine | 400 | 59.0 |
| | 1200 | 67.6 |
| | 2000 | 52.3 |
| 2-Thiocytosine | 400 | 66.1 |
| | 800 | 65.5 |
| | 1200 | 69.5 |
| 2-Thiouracil | 50 | 53.3 |
| | 100 | 38.6 |
| | 200 | 26.8 |
| Experiment 2 | | |
| Control | | 200 |
| 2-Thiouracil | 10 | 147 |
| | 50 | 147 |
| | 100 | 120 |
| Benzimidazole | 100 | 204 |
| | 500 | 188 |
| | 1000 | 156 |
| 6-Nitrobenzimidazole | 100 | 192 |
| | 300 | 204 |
| | approx. 500 | 64 |

* The test compounds were applied onto leaves as a spray. In experiment 1, one preinoculation and two postinoculation applications were made; in experiment 2, two applications were made daily after inoculation.

** The numbers of local lesions are expressed as a mean of 8 leaves for experiment 1 and of 20 leaves for experiment 2.

Table 7. Effect of substituted purines, pyrimidines and related compounds on a sweet potato strain of tobacco mosaic virus in detached leaves of N. glutinosa

| Test Compounds | Treatment Concentrations mg./l. | Mean no. of local lesions on 2 half-leaves treated / control: 2 days after inoculation | 4 days after inoculation |
|---|---|---|---|
| * 8-Azaadenine | 200 | 0 / 31 | 2 / 38 |
| ** 8-Aza-2,6-diaminopurine | 200 | 1 / 35 | 7 / 40 |
| ** 8-Azaguanine | 200 | 11 / 21 | 23 / 24 |
| 8-Azahypoxanthine | 200 | 35 / 28 | 39 / 32 |
| 8-Azaxanthine | 200 | 10 / 15 | 21 / 20 |
| 8-Chloroxanthine | 150 | 16 / 14 | 25 / 20 |
| * 2,6-Diaminopurine sulfate·½ $H_2O$ | 200 | 0 / 25 | 0 / 38 |
| 6-Oxy-2,8-dithiopurine | 200 | 22 / 27 | 25 / 32 |
| * 6-Methylpurine | 10 | - / 28 | - / 28 |
| 6-Chloropurine | 200 | 16 / 21 | 24 / 23 |
| * 2-Thioadenine | 200 | - / 26 | - / 26 |
| 6-Amino-4-oxy-2-thiopyrimidine | 200 | 15 / 11 | 17 / 16 |
| 2-Amino-4-oxy-2-thiopyrimidine | 200 | 30 / 24 | 32 / 34 |
| 5-Bromouracil | 200 | 25 / 41 | 30 / 42 |
| * 4,5-Diamino-6-oxy-2-thio-pyrimidine | 150 | 20 / 25 | 16 / 27 |
| * Diazouracil | 200 | 14 / 22 | 16 / 22 |
| 5-Methyl-4-oxy-2-thiopyrimidine | 200 | 0 / 15 | 12 / 18 |
| 6-Methyl-4-oxy-2-thiopyrimidine | 200 | 12 / 13 | 13 / 14 |
| 5-Nitrouracil | 200 | 16 / 21 | 16 / 23 |
| 2-Thiouracil | 200 | 0 / 19 | 0 / 19 |
| Benzimidazole | 200 | 11 / 28 | 24 / 31 |
| * 6-Nitrobenzimidazole | 200 | 1 / 45 | 5 / 51 |
| * 2,4-Dithiopyrimidine | 150 | - / 34 | - / 35 |

* Phytotoxicity occurred.

** Compounds were dissolved in 0.1 % $NaHCO_3$.

\- No local lesions produced on the treated leaves which became necrotic or water-soaking due to the treatments.

Of compounds tested on N. tabacum, 6-methylpurine produced the most severe phytotoxic effect. Four applications of this compound at 50 mg./l. caused stunting of the tobacco seedlings accompanied by yellowing and down-curling of young leaves. Top necrosis usually resulted within a week after cessation of the treatment. The compound also caused yellowing and stunting in mature N. glutinosa plants. 5-Nitrobenzimidazole at 500 mg./l. and 2-thiouracil at 100 mg./l. and 200 mg./l. caused yellowing of young leaves, curling down of leaf margins and retardation of apical growth in both N. glutinosa and N. tabacum. Benzimidazole at 1000 mg./l. caused formation of small, white, necrotic, slightly raised spots on leaves, especially young leaves, of N. glutinosa. Plants receiving 8-azaguanine at 1200 mg./l. became lighter in color than the untreated. No local injury was observed. Phytotoxicity taking the form of small white spots on young leaves of N. tabacum was also noted for 5-bromouracil at 500 mg./l.

It should be emphasized that phytotoxic effects of a compound on growing plants is not always similar to that observed with detached plant tissues. Thus, Commoner and Mercer (2) and Schneider (38) reported no injurious effect of 2-thiouracil, 2,6-diaminopurine and 8-azaadenine on cultured leaf-discs of tobacco, while Matthews and Smith (26) found severe damage on growing plants caused by these compounds. It was the author's observation that 2-thiouracil at 200 mg./l. when applied to growing N. glutinosa plants caused stunting and leaf yellowing, but had no visible effect on leaves floated in solution containing the compound. On the contrary, 2,6-diaminopurine, 6-nitrobenzimidazole and 8-azaadenine were more phytotoxic on N. glutinosa when applied to detached leaves than to growing plants.

### Tests With Acti-diones on Wheat Streak Mosaic Virus

The effect of Acti-dione and its three derivatives has been tested against wheat streak mosaic virus in wheat and results are given in Table 8. All compounds sprayed at 10 ppm caused no suppression of symptom expression. Acti-diones T and M were also ineffective at 50 and 100 ppm. A delaying effect was observed with Acti-dione and Acti-diona S at concentrations of 50 and 100 ppm. The treated plants, however, suffered seriously from the phytotoxicity of the compounds (or their carrier). Acti-diones S, T, and M at 50 and 100 ppm caused some of the central rolled leaves to break at the basal portion. In this case the systemic symptoms on the plants could only be observed after the appearance of new tillers. The Acti-dione treated plants generally were lighter colored than the controls. This rendered difficult the early detection of diseased plants. It is probable that the toxic effect of the test compounds on the plants accounted in large part for the apparent delay in symptom development. Because of the phytotoxicity on wheat, studies with these compounds were terminated.

### Tests With Plant Growth Regulators on Wheat Streak Mosaic Virus

The plant growth regulators tested against wheat streak mosaic virus were 2,4-dichlorophenoxyacetic acid, naphthaleneacetic acid and indolebutyric acid. They were applied to wheat plants either through the soil or onto leaves as a spray. Inoculation either followed the application or was done before it. Table 9 summarizes the results obtained.

Phytotoxic effects were observed with 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid at 10 mg./100 ml. or above. At these concentrations both compounds caused wilting and serious leaf burning. Treated plants

Table 8. Effect of Acti-dione and its three derivatives on wheat streak mosaic virus in wheat.*

| Test Compounds | Treatment Concentration (ppm) | No. days taken to show symptoms, mean of 25 plants | Apparent Incubation period lengthened in percentage |
|---|---|---|---|
| Control | | 7.40 | |
| Acti-dione** | 100 | 9.83 | 32.8 |
| | 50 | 8.76 | 18.4 |
| | 10 | 7.72 | 4.3 |
| Acti-dione T*** | 100 | 7.84 | 6.0 |
| | 50 | 7.55 | 2.0 |
| | 10 | 7.08 | -4.3 |
| Acti-dione S*** | 100 | 9.08 | 22.7 |
| | 50 | 8.68 | 17.3 |
| | 10 | 7.56 | 2.2 |
| Acti-dione M*** | 100 | 8.08 | 9.2 |
| | 50 | 7.24 | 2.2 |
| | 10 | 7.08 | -4.3 |

* Since the Acti-diones used are commercial preparations in liquid, dilution was made according to accompanying directions. The concentrations indicated in the table are based on the content of the active ingredient rather than the liquid itself.

The test compounds were sprayed onto leaves by means of an atomizer. Two applications on consecutive days began the next day after inoculation.

** Acti-dione treated plants took a lighter color than the controls; the early detection of diseased plants was thus rendered difficult.

*** Acti-diones S, T, and M at 50 and 100 ppm caused some of the central rolled leaves to break at the basal portion.

Table 9. Results of tests on the effect of three plant growth regulators on wheat streak mosaic virus in wheat.*

| Test Compounds | Concentration mg./100 ml. | Chemicals applied as a soil drench: Applications made before inoculation: No. of plants treated | No. of plants showing symptoms | No. of plants died before record taken | Applications made after inoculation: No. of plants treated | No. of plants showing symptoms | No. of plants died before record taken | Chemicals applied as a spray: Applications made before inoculation: No. of plants treated | No. of plants showing symptoms | No. of plants died before record taken | Applications made after inoculation: No. of plants treated | No. of plants showing symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | | 10 | 10 | | 10 | 10 | | 10 | 10 | | 10 | 10 |
| 2,4-Dichlorophenoxyacetic acid | 10** | 12 | 5 | 7 | 12 | 5 | 7 | 12 | 12 | | 9 | 9 |
| | 1 | 12 | 12 | | 12 | 11 | 1 | 11 | 11 | | 10 | 10 |
| | 0.1 | 11 | 11 | | 12 | 12 | | 12 | 12 | | 12 | 12 |
| Naphthaleneacetic acid | 10** | 12 | 8 | 4 | 12 | 12 | | 11 | 11 | | 12 | 12 |
| | 1 | 9 | 9 | | 12 | 12 | | 10 | 10 | | 10 | 10 |
| | 0.1 | 12 | 12 | | 11 | 11 | | 12 | 12 | | 12 | 12 |
| Indolebutyric acid | 10 | 11 | 11 | | 11 | 11 | | 12 | 12 | | 12 | 12 |
| | 1 | 12 | 12 | | 12 | 12 | | 12 | 12 | | 11 | 11 |
| | 0.1 | 12 | 12 | | 11 | 10 | 1 | 11 | 10 | 1 | 11 | 11 |

* Five daily applications of compounds were made in each case.

** Phytotoxicity occurred.

leaves might be expected after leaf rubbing. This treatment, however, enhanced the phytotoxicity of 2-thiouracil, and did not increase the inhibitory effects of any of nine compounds tested. It, therefore, seemed more likely that the ineffectiveness of these compounds as chemotherapeutants of cereal viruses in wheat was a property of the virus and the compounds concerned rather than the result of inadequate absorption.

As revealed in tests with Datura stramonium L. and wheat, the effect of 8-azaguanine on brome grass mosaic virus may be exhibited in three ways: (1) decreasing the number of successful infection points, (2) depressing virus multiplication and (3) delaying the movement of the virus away from inoculated leaves. Perhaps, these effects could be enhanced if this compound were applied to less susceptible varieties and was more adequately absorbed. For a compound such as 8-azaguanine which possesses a fairly strong virus inhibitory activity and which enters into treated plants in small amount owing to low solubility, slow multiplication and development of the virus in a less susceptible host might allow better chance for the compound to show its maximum action. To improve absorption of 8-azaguanine through combination with other compounds has not been attempted. With antibiotics Gray (7) found that the addition of about one percent glycerol to streptomycin solution caused several-fold increase of the amount of the antibiotic absorbed by bean leaves. Similar possibilities might exist for 8-azaguanine also.

Results of tests with a sweet potato strain of tobacco mosaic virus confirm the observations of Mercer et al. (28) and Schneider (38) that 2-thiocytosine and 2,6-diaminopurine inhibit development of the virus. But, Matthews and Smith (27) made different observations. According to them the delaying effects on systemic virus development of 2,6-diaminopurine as a spray could not be dissociated from its inhibitory effect on plant growth.

They also found no detectable effect of 2-thiocytosine on systemic development of tobacco mosaic virus. This contradiction can possibly be accounted for by the different experimental conditions and certainly indicates the need for much more careful work in this field.

Two compounds, never studied previously, 8-aza-2,6-diaminopurine and 5-methyl-4-oxy-2-thiopyrimidine have been found to be somewhat inhibitory, although not so effective as 2-thiouracil, to local lesion formation by tobacco mosaic virus in detached N. glutinosa leaves. It might be interesting to point out that 8-aza-2,6-diaminopurine is structurally similar to 8-azaguanine and 2,6-diaminopurine both of which are virus inhibitory compounds. It might also be of interest to note that 5-methyl-4-oxy-2-thiopyrimidine finds its structural similarity in thymine rather than uracil. What the deeper meanings associated with these observations are remain to be determined.

## SUMMARY

Twenty-four substituted purines, pyrimidines and related compounds have been tested for their possible inhibitory activities against wheat streak mosaic virus, barley stripe mosaic virus, brome grass mosaic virus and a sweet potato strain of tobacco mosaic virus. One compound, 8-azaguanine as a leaf spray, was found to delay the expression of symptoms caused by brome mosaic virus in wheat. This compound also reduced the number and inhibited the development of local lesions caused by brome grass mosaic virus on Datura stramonium. All compounds tested either had little or no effect on either wheat streak mosaic or barley stripe mosaic virus symptom development.

Results of tests with a sweet potato strain of tobacco mosaic virus

confirmed the inhibitory activities of 2-thiouracil, 2-thiocytosine, 8-aza-guanine, 8-azaadenine, 2,6-diaminopurine, 6-chloropurine, benzimidazole and 6-nitrobenzimidazole as indicated by previous investigators. Two hitherto unreported compounds, 8-aza-2,6-diaminopurine and 5-methyl-4-oxy-2-thio-pyrimidine, were found to inhibit local lesion formation caused by tobacco mosaic virus in detached Nicotiana glutinosa leaves.

Tested against wheat streak mosaic virus in wheat three plant growth regulators and four Acti-diones were found ineffective as anti-viral agents below phytotoxic concentrations.

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# PARTIAL CHEMOTHERAPY OF THREE CEREAL VIRUSES AND TOBACCO MOSAIC VIRUS WITH CERTAIN ANALOGUES OF PURINE AND PYRIMIDINE AND SEVERAL OTHER ORGANIC COMPOUNDS

by

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The study of plant virus chemotherapy has produced some spectacular results in the last decade. A great number of compounds are now known as inhibitory to virus multiplication. Some when applied to growing plants are able to delay or completely suppress symptom expression. As a group, analogues of purine and pyrimidine, antibiotics, and plant growth regulators have been most widely studied and their effectiveness most well-established.

It was the purpose of this study to seek for compounds which might be inhibitory to cereal viruses. Three manually transmissible viruses, e.g. wheat streak mosaic virus, barley stripe mosaic virus, and brome grass mosaic virus were used. One strain of tobacco mosaic virus (sweet potato) was also employed, largely for the purpose of confirmation of previous work and techniques.

The wheat variety Marquillo-Oro-Pawnee selection no. 462666 was used as the systemic host of all three cereal viruses. Datura stramonium L. was employed as another host of brome grass mosaic virus. When inoculated the plant produced etched rings on leaves with normal green or light green islands at the center. This local lesion response of Datura to the brome mosaic virus was found in the course of this study. Nicotiana tabacum variety Havana 38 and N. glutinosa were used as hosts of the strain of tobacco mosaic virus.

Compounds tested included 24 analogues of pyrimidine and purine, three plant growth regulators and four antibiotics known as Acti-diones. In most cases the compounds were applied to plants as leaf sprays, but on some occasions they were applied as soil drenches or leaf dips.

The testing of the plant growth regulators and Acti-diones was confined to wheat streak mosaic virus in the early part of the study. They had no effect or only negligible effects on the virus below phytotoxic concentrations and were so phytotoxic that work with them was discontinued.

Of the purine and pyrimidine analogues tested, 8-azaguanine as a leaf

spray markedly delayed the symptom development caused by brome grass mosaic virus in wheat plants. The treated plants stood out distinctly from the controls because of their milder mottling and deeper green color. Watered on the soil, however, 8-azaguanine was ineffective. With wheat streak mosaic virus a slight increase in incubation period, corresponding to the increase of concentrations of the test compounds, was noted for plants treated with 8-azaguanine, 2,6-diaminopurine, 2-thiouracil, benzimidazole, 6-nitrobenzimidazole and 6-chloropurine. However, the effect, although consistent, was too slight to be of certain significance. All compounds tested lacked any inhibitory effect against barley stripe mosaic virus.

Applied to Datura stramonium, 8-azaguanine decreased local lesions caused by brome mosaic virus and delayed their formation. Reduction of lesion numbers was also observed with plants receiving 8-azaadenine, 6-amino-4-oxy-2-thiopyrimidine and other compounds. Since the local lesion reaction of Datura stramonium was normally rather erratic, tests are planned for the future to affirm the results already obtained.

In experiments with the sweet potato strain of tobacco mosaic virus, the effectiveness of 2-thiouracil, 2-thiocytosine, 8-azaadenine, 2,6-diaminopurine, benzimidazole and 6-nitrobenzimidazole as virus inhibitory substances was confirmed. Two compounds, 8-aza-2,6-diaminopurine and 5-methyl-4-oxy-2-thiopyrimidine, which have not been reported before were found to inhibit local lesion formation in N. glutinosa caused by tobacco mosaic virus when applied to detached leaves in petri dishes.

Phytotoxic effects were observed with 6-methylpurine on all test plants; 2-thiouracil, benzimidazole, 6-nitrobenzimidazole and 6-chloropurine on Datura plants; 2-thiouracil and 6-nitrobenzimidazole on Nicotiana tabacum and N. glutinosa; and 2-thiouracil on wheat plants.