Research
FOR FARMERS

SUMMER — 1959

CANADA DEPARTMENT OF AGRICULTURE

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CANADA DEPARTMENT OF AGRICULTURE
Ottawa, Ontario

HON. DOUGLAS S. HARKNESS, *Minister* — J. G. TAGGART, C.B.E., *Deputy Minister*

NOTES AND COMMENTS

The advantages of using good seed are so obvious that one wonders why it is not a universal practice. Hazards of crop production are serious enough under the most favorable conditions and to sow anything but the best quality seed obtainable is certainly less than wise. But it is not enough to stress the physical quality of the seed; it must be of the right variety or strain, adapted to the needs of the market and the conditions of the environment. This is illustrated by a recent three-year test at the Lennoxville station which showed that Canadian varieties of timothy gave substantially better yields of dry matter than American or British varieties. Sometimes this superiority of variety or strain is limited to a narrow area so that what is best in one locality may not excel in the next county or even in the next field.

* * *

Farmers are generally quick to notice any changes in the appearance of their crops. Sometimes these changes merely indicate good, rapid growth under the best of conditions. Again, they may reveal a lack of moisture, of nutrients, or the presence of insects or plant diseases. But not all the significant changes occur where they may be readily observed. Within the soil itself, innumerable microorganisms thrive or otherwise as conditions dictate, and these may have a profound influence on crop growth. The relationship between microorganisms and crops is explained by Drs. Katznelson and Rouatt in this issue.

* * *

Mechanical grazing of irrigated pastures may provide one of the answers to the problem of cheaper beef production. In a feeding trial in southern Alberta conducted by the P.F.R.A. on 17 acres of irrigated pasture, 40 steers made average daily gains of 2.21 pounds to produce over 600 pounds of beef per acre from grass alone. After deducting all expenses including cost of seed, labor, machinery charges and interest on investment, net profits of $69 per acre were realized.

* * *

Consciously or otherwise, farmers rely heavily on the law of probability in planning their seasonal operations. Few things are as uncertain as the weather but the probable occurrence of certain weather phenomena can be predicted fairly accurately on the basis of long-time records. Such data recently published for the Ottawa area, show the percentage probability of late spring and early fall frosts for any given date. Armed with this information, a grower can determine safe planting and harvesting dates for tender crops and at the same time estimate the odds of successfully avoiding damage from earlier planting or later harvesting in an attempt to gain market advantage. It may be some time before similar information is compiled for all areas of the country but the meteorologists are working at it.

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Cover Photo—Dr. H. Katznelson (left), Director, Microbiology Research Institute, Ottawa, and associate Dr. J. W. Rouatt, discussing the influence of microorganisms (isolated from roots) on plant nutrition and disease. *Inset:* Close-up of fungal colonies Dr. Rouatt was counting on plate at time photo was taken. (See story on p. 8.)

The Search for Phosphate Insecticides of Low Hazard

R. D. O'Brien

THE first phosphorus insecticides were patented by Schrader in Germany in 1937. These compounds were of extreme hazard to mammals, as must have been at once evident, for Schrader's patents were kept secret and on their basis a great deal of research was done to develop compounds even more lethal to humans. When the war was over, the full excellence of the German research was revealed. They had indeed developed some terrible "nerve gases"—tabun, sarin and soman, of which tabun was being made at 100 tons a month. But several useful insecticides also emerged, the best known being parathion and TEPP. Although lacking the apalling hazard of tabun, these compounds were about as toxic to mammals as to insects. However, their dramatic effectiveness in the field overcame any reluctance to handle such dangerous compounds, and now about 20 million pounds of parathion and its relative, methyl parathion, are made annually in the world.

Naturally the manufacturers have been anxious to find safer compounds. The first important one was malathion (1950) which was followed by Diazinon and Chlorthion. Within the last few years several compounds have been produced with such a good safety factor that they can be used for control of internal insect parasites in livestock. Co-Ral and Trolene are already in use for this purpose, and several others are being developed. Nevertheless, of the popular compounds, it is estimated that about 22 million pounds of hazardous as against 12 million pounds of "safe" compounds are produced annually in the world.

All the new "safe" compounds were obtained by extensive synthesis and screenings. One of the facts that has emerged has been that a fantastic variety of substituents can be attached to the phosphorus atom. About 50,000 compounds have already been tested. There is thus reason to presume that many valuable new compounds could be made. In order to find them, is there an alternative approach to this semi-random mass synthesis and screening program? I think so. If we can find out precisely why the "safe" compounds are so, we can hope to *design* new compounds.

In this attempt, we have at first restricted our interest to finding why some compounds kill insects but not mammals, and it is in that sense that we shall talk below of "selectivity". Six steps constitute the picture of poisoning (Figure 1). The physiological or biochemical consequences to an attacked target must also be considered, as well as the possible storage of the compound or its toxic metabolites in the tissues. If a compound kills an insect but not a mammal, it must be because one (or more) of these steps is different.

All the selective organophosphates we have studied are selective when injected into, rather than applied in other ways to the animals; therefore the differences do not arise in steps 1 or 2. Numerous experiments have convinced us that in insects and mammals the target is the same: the vital nerve enzyme called cholinesterase. We therefore feel sure that selective toxicity must be due to a difference in one or more of the steps 3, 4, 5 and 6.

With few exceptions, all the strongly selective organo-phosphates contain a group P=S, and these compounds are invariably transformed by enzymes in animal bodies to the P=O derivatives; these are the active metabolites which actually kill the animal, and this oxidation of P=S to P=O is called 'activation'. The animal therefore takes a part in poisoning itself. However, all animals have other enzymes that can break down organophosphates to non-toxic products: this is degradation. The factors that make an organophosphate containing P=S toxic to a given animal are (a) a P=O derivative which is a potent inhibitor of cholinesterase and can reach the target; (b) a balance of activating and degrading enzymes and of excretion

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Dr. O'Brien is a specialist in comparative toxicology in the Department's Pesticide Research Institute, London, Ont.

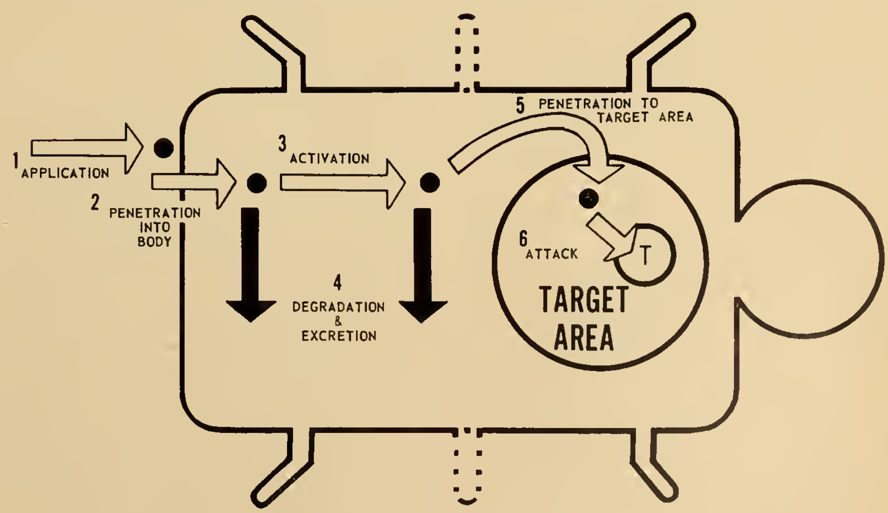


Figure 1. Possible steps involved in poisoning of any animal by any compound.

which permits a high and persistent level of P=O derivative. If such an organophosphate is toxic to insects, it is probable that the P=O derivative inhibits cholinesterase well. Our knowledge of the insect nervous system, wherein lies the target enzyme, cholinesterase, tells us that it is much better protected than that of the mammal: there seem to be no cases of compounds that can penetrate to the insect and not the mammalian nerve. Therefore condition (a) is satisfied on both counts whenever we are studying compounds toxic to insects, and it must be in (b) that the crucial difference between insects and mammals lies. This could involve steps 3 and 4 and perhaps storage effects.

Our first experiments (1957) showed that when we added malathion to tissues removed from insects and from mammals, both activation and degradation could be shown. But with insect tissues, activation outstripped degradation, and the P=O derivative (called malaoxon) accumulated. In mammalian tissues, degradation outstripped activation, and no malaoxon accumulated. A similar effect was found with Co-Ral. This year H. R. Krueger and I have demonstrated that the same is true in intact animals, by using radioactive malathion. Following malathion injection, very little malaoxon accumulated in the whole mouse (Figure 2), but very large levels of malaoxon were produced and persisted in the American cockroach. We have also shown this with the house fly and the German cockroach, and we find precisely the same picture with the other selective insecticides we have studied: Dimethoate, Diazinon and acethion. We feel that such a pattern accounts entirely for the selective toxicity of these compounds.

Are these striking differences caused by differences in activation, degradation or storage? Degradation is easiest to study, so we examined it first. For instance, mammalian tissues rapidly degrade added malaoxon, but insect tissues are very poor in this respect. Several other approaches suggest to us that in

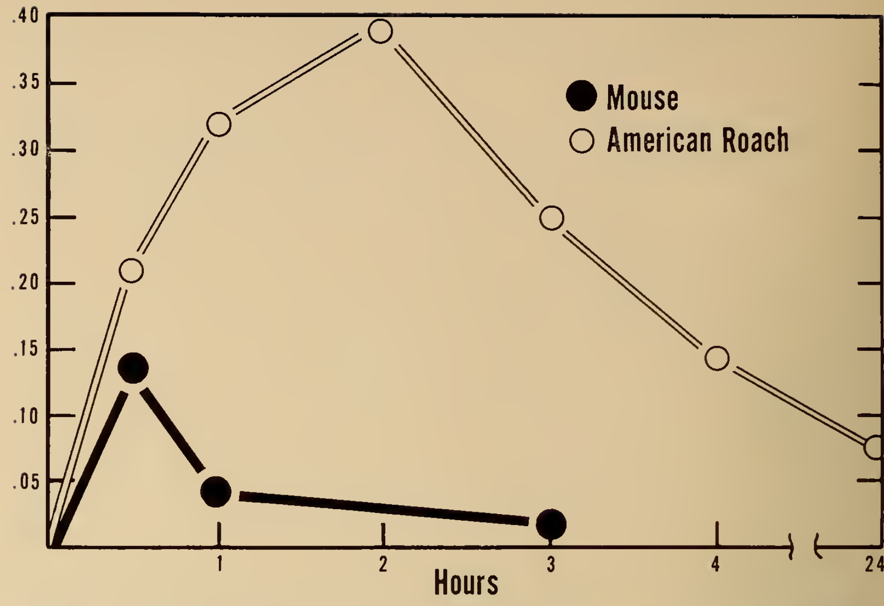
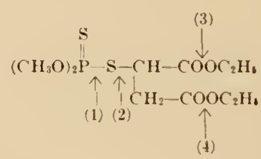


Figure 2. Levels of malaoxon (the P=O derivative) after injection of 30 mg./kg. of malathion (P=S compound) into mouse and cockroach. Levels are in micrograms per gram body weight.

most cases it is in degradation that the important differences occur.

It is true for nearly all organophosphates that degradation is hydrolytic (i.e. involves splitting by water, usually assisted by an enzyme), and that hydrolysis always degrades. This is a vital link in our argument. Now hydrolysis of organophosphates can be produced by enzymes of two kinds: phosphatases, and others. The phosphatases break the molecule close to the phosphorus atom, the others at more distant points. Thus malathion can be broken at four places, shown by arrows:



The phosphatases act at (1) and (2). We find that the reason that the mammal degrades malathion so well is that the enzymes acting at (3) and (4) are extremely active; they are very weak in the insect. We then argued that these particular enzymes could not be uniquely active on malathion, which is an exotic compound to animals; it must be effective on other compounds also. Therefore if we build the group $COOC_2H_5$ into other organophosphates, mammals should be able to degrade such compounds more readily than insects, and thus should make the organophosphate selective. We designed such compounds. They were selective.

Of the six new compounds we made the best was acethion: $(C_2H_5O)_2P(S)SCH_2COOC_2H_5$, which was rather more lethal than malathion to flies and considerably less lethal to mammals than malathion.

We can now suggest the "principle of the susceptible group": certain kinds of chemical groups are particularly susceptible to hydrolysis (and therefore degradation) in mammals, but less so in insects. Such groups should bestow selectivity upon organophosphates into which they are introduced. Our business must be to find more of these groups, with the eventual aim of being able to "build in" selectivity to any organophosphate which has otherwise desirable properties.

This may not be the only way to achieve selectivity. We are studying carefully the differences between mammalian and insect cholinesterase, hoping to take advantage of any opportunity to design compounds selective for the insect enzyme. Perhaps differences exist in activating systems. Our aim is to show that the most effective approach to new selective agents is rational, not random.

Bovine Vibriosis in British Columbia

P. L. Stovell AND *R. J. Avery*

Top: Visual examination of bovine cervix with an illuminated plastic speculum. *Middle:* Method of placing the cellulose sponge tampon in the forward part of the vagina for collection of cervicovaginal secretion. *Bottom:* Method of withdrawal of the loaded sponge tampon.

THE agent of vibriosis, a bacterium known as *Vibrio fetus*, has its natural habitat in the sheath of the bull where it causes no discernible adverse effect. Bulls may remain infected for many years and although effective treatments are available re-infection may occur with great ease and with no outward symptoms.

Cows may be infected through natural service or through artificial insemination with untreated semen. The organism infects the uterus and sets up a chronic inflammation that leads to the early death of the fertilized egg, or more rarely to abortion in the later stages of pregnancy. After vibriosis has been present in the herd for a few months increasing numbers of cows will carry their calves to term although some may still be infected. Some cows remain carriers of the disease for long periods. This changing herd picture is a result of the local development in the genital tract of antibodies that can be detected by the laboratory test with cervicovaginal mucus.

At our laboratory we have been concentrating on the diagnostic aspects of vibriosis so that infection may be reliably detected in the females on a herd basis. The mucus agglutination test is widely accepted for this purpose, but it must always be interpreted on the basis of the herd and not the individual animal. We are also working on the development of a reliable and economically practical diagnostic method for bulls. Since the bull supplies the natural habitat for the organism, such a test is fundamental to the final eradication of the disease.

Two primary obstacles have to be overcome. In the first place the organism is often present in the male sheath in extremely small numbers. This is also the case in partially immune carrier cows. Secondly, the bull sheath contains a heavy flora of other microorganisms that not only aggressively outgrow the fragile *Vibrio fetus* organisms on culture media but also render the environment untenable for them.

In the past by far the greatest part of the economic loss from vibriosis has preceded the diagnosis, but this need no longer be the case if the cattle owner consults his veterinarian as soon as he suspects the disease in his herd. As always with infectious diseases and especially with those producing infertility in cattle, early diagnosis is of the utmost importance. For this purpose, a fresh or fresh-frozen aborted fetus provides the best source for the isolation of the organism. Since this is seldom available, a herd breeding history along with samples of cervicovaginal secretion from selected animals should be submitted immediately by the veterinarian. At the same time, the veterinarian will attempt to determine the type of infertility present and collect whatever additional specimens are needed.

The experience of a cattle rancher in British Columbia illustrates clearly the economic losses that may result from vibriosis. In April 1958 we received a report of a distinctly abnormal calving picture in one of his three beef herds. This herd of 142 predominantly Hereford cows, aged five to seven years, had entered the pasture the previous spring with 143 calves at foot, including one set of twins. Early in June four mature sires were introduced into the herd, and gave every indication of activity up to the time they were removed September 20.

Dr. Stovell is officer-in-Charge of the Animal Pathology Laboratory, Vancouver, B.C., and Dr. Avery is in charge of the A.P. Lab., Lethbridge, Alta.

Three weeks after the expected calving date, only six cows had calved, and not more than twelve others were showing signs of early freshening. We immediately forwarded to the veterinarian materials for sponge-tampon collection of cervicovaginal secretions for laboratory agglutination tests. Of the 20 animals tested, 19 showed significant levels of antibody for the *Vibrio fetus* organism in the genital secretions.

At the end of the sixth week of the expected calving period, only nine more cows had calved, bringing the total to 15 or 10.6 per cent. In the tenth week all but the 30 cows that had calved or were obviously close to calving, were sold for slaughter. The genitial tracts of these 112 cows were examined and 41 were found to be pregnant. The maximum calf crop could not therefore have exceeded 71 or 50 per cent. Of these 71 calves, 75 per cent were or would have been born in the latter half of the calving period, and 50 per cent during the last quarter. The breeding period lasted 95 days so that the conception data can be summarized approximately as follows:

	Cumulative Total %
Following first oestrual period	4.0
Following second oestrual period	10.6
Following third and fourth oestrual period	21.6

* * *

Vibriosis was first diagnosed in B.C. in 1951 in the Fraser delta. Since then it has become widespread in the lower mainland of British Columbia and on Vancouver Island.

* * *

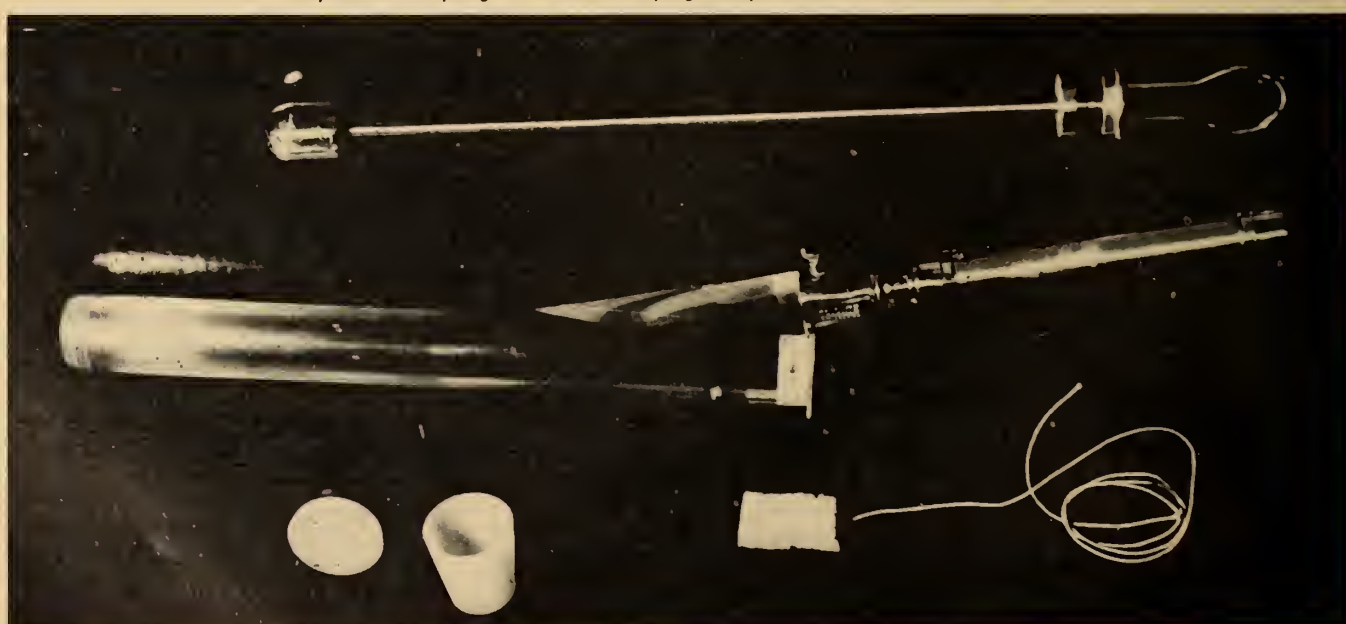
This relative improvement in the conception rate at the end of the breeding season suggested an infectious form of infertility. Semen viability and working ability of the bulls in the early part of the season could hardly be questioned when it was so well demonstrated at the end of the period. The picture was in fact characteristic of vibriosis in a herd not previously exposed to the disease and without immunity to the infection.

The next step was to investigate the most likely source of vibriosis infection. Few outside additions of beef breeding stock had been made on this ranch and these had all been to the other two beef herds that were calving normally. On the same ranch, however, was a small diary herd to which a mature bull and five bred heifers had been introduced from premises 300 miles away where vibriosis had been diagnosed six years before. These six animals had arrived at the ranch just two months prior to the introduction of the bulls to the pasture. Agglutination tests were made and antibodies for *Vibrio fetus* detected in cervicovaginal secretions in eight of these thirteen dairy animals, indicating that vibriosis was or had been present. This seemed to be strong circumstantial evidence that the dairy animals represented the source of the infection since the management of the ranch was otherwise very good.

This is an example of the rapidity of spread by natural breeding, the diminution of the calf crop, and the setting back of the calving date. It demonstrates how easily one error in herd management can lead to serious economic losses and strongly supports the recommendation that the importation and maintenance of cattle should be accompanied by strict isolation, and that no natural service should be allowed. On this particular ranch in the 1958 breeding season, the other two beef herds and the dairy herd were bred exclusively by artificial insemination. Early indications are that the conception rates have been good.

It is fortunate for the dairy industry that artificial insemination with antibiotic-treated semen has been developed in time to aid in the control of vibriosis. The administration of effective antibiotics is helping to lower the incidence and clinical manifestations of the disease, but the importance of early diagnosis cannot be overemphasized.

Speculum and plunger with cellulose sponge tampon attached to withdrawal cord.



Left: Swathing grain plots for kernel moisture sampling. *Above:* Weighing grain samples for kernel moisture determination.

SWATHING STUDIES

Behavior of Standing and Swathed Grain During Natural Drying Process Revealed

M. E. Dodds

SWATHING STUDIES, conducted over a five-year period at the Experimental Farm, Swift Current, Sask. indicate that barley may be cut at a kernel moisture content of 40 per cent and wheat at 35 per cent without significant loss of bushel weight or yield. Barley kernels reached 40 per cent moisture content from nine to eleven days before the crop would be considered suitable for straight combining. Similarly, wheat could be swathed at 35 per cent moisture seven to nine days earlier than it could be safely combined. Farmers would be well advised to swath their grain at these earlier stages because swathed grain is much more protected than standing grain against natural losses caused by rain, hail, wind, insects or frost. Daily observations of moisture content of standing and swathed grain revealed that the swath could be recovered with a combine four to six days after cutting. Standing grain dried at a somewhat slower rate.

A variety of weather conditions was encountered throughout the five harvest seasons of the period under study. Two of these years in particular illustrate the extremes that are experienced in Western Canada at this time of year and are used in the following discussion to explain the behavior of grain during the natural drying process.

In 1954 the start of harvesting was delayed because of adverse weather conditions, and field operations were interrupted by intermittent showers in the evenings. The days, however, were warm and bright. Rains, periods of high humidity, and of sunshine were reflected in fluctuations in the kernel moisture content of the standing and swathed grain but both barley and wheat continued to dry and mature.

The grain in the swath gained more moisture than standing grain during the intervals of damp weather but dried faster when less humid conditions prevailed. Swathed grain lost moisture more rapidly than standing grain during any given period. Near the end of the 1954 tests, because of the weather conditions, standing and swathed grain reached the equilibrium moisture content at about 19 per cent. The rate of absorption and drying was approximately the same for both at this stage and subsequent maturing progressed at a uniform rate. It is at this stage of maturity that standing grain appears uniformly ripe and the tendency of most farmers is to wait for this condition before swathing. However much risk could be avoided if the crop were swathed a week or more earlier as pointed out above.

In 1955 ideal harvest weather prevailed and swathing was not delayed by rain or poor weather conditions. Cutting was started at an early stage of maturity with both barley and wheat and was continued without interruption

(Concluded on page 10)

The author is a specialist in harvesting equipment at the Experimental Farm, Swift Current, Sask.

Interrelationships between Soil Microorganisms and Plants

H. Katznelson AND *J. W. Rouatt*

The influence of inoculation with nodule bacteria on the growth of peas; this is an excellent example of symbiotic fixation of atmospheric nitrogen.

MICROORGANISMS, like other forms of life, respond vigorously to an increased food supply and so the addition of manures to soil causes rapid multiplication of a host of organisms such as bacteria, actinomycetes, fungi, and protozoa. After this microbial bloom, when the food has been consumed and when competitive forces among the organisms themselves have asserted their influence, the number and relative proportions of these forms return more or less to the levels that were characteristic of the soil prior to the addition of manure.

Insofar as the soil microbial population is concerned, the planting of a seed followed by the penetration of the soil by the root is equivalent to adding food to the soil. The plant, of course, draws on the soil for nutrients in competition with the microflora but it apparently gives more than it takes. The germinating seed liberates appreciable quantities of sugars and amino acids into the soil solution and the growing root excretes these and other substances. Dead and dying tissues sloughed off by the root also constitute a source of nutrients for soil microorganisms. In response to this supply of readily available food, the soil microflora near the root and on the root surface begins to multiply rapidly and because of the large increase in numbers of organisms in this region associative and antagonistic effects among them come into sharp focus. There results finally an unstable balance or equilibrium among these forms in which certain types predominate and others are inhibited and which remains essentially unchanged as long as the plant is actively growing and environmental conditions are undisturbed. The stimulation of microbial growth and activity at and near the root surface and the effect of this microbial development on the growth and health of the plant is known as "the rhizosphere effect".

The authors are with the Microbiology Research Institute, Research Branch, Ottawa. Dr. Katznelson is Institute Director and Dr. Rouatt is head of the Plant Microbe Interrelationships Section.

There is no lack of data showing an increase in numbers of various groups and types of organisms on the roots of a variety of plants. Such data for wheat are shown in the table. It may be noted that not all groups are increased in the rhizosphere; nitrifying bacteria, Azotobacter, spore-forming bacteria, and algae do not multiply extensively and the latter group is rather severely inhibited. With other plants and in different soils there may be over 100 times as many bacteria in the rhizosphere as in the surrounding soil and counts of 5 to 10 billion per gram of soil are not uncommon. Increases in other groups of organisms (fungi, protozoa, etc.) are more modest.

The legume bacteria produce nodules on the roots of leguminous plants and this association results in the fixation of atmospheric nitrogen which the plant utilizes.

Perhaps even more important than the numerical changes in the rhizosphere are the changes in the types of organisms developing in it. The roots cause a significant shift in the proportions of certain groups of bacteria, fungi, and other

TABLE I

NUMBERS OF DIFFERENT GROUPS AND TYPES OF ORGANISMS IN RHIZOSPHERE OF WHEAT AND IN NON-RHIZOSPHERE SOIL (PER GRAM DRY SOIL)

Group	Rhizosphere soil	Non-rhizosphere soil
Total bacteria	1,120,000,000	53,000,000
Fungi	1,160,000	120,000
Protozoa	2,410	990
Algae	4,500	27,000
Nitrifying bacteria	100,000	100,000
Spore-forming bacteria	930,000	575,000
Aerobic cellulose decomposers	720,000	2,700
Anaerobic cellulose decomposers	120,000	9,100
Gas-producing anaerobes	389,000	33,000
Anaerobic bacteria	12,000,000	6,500,000
Ammonifying bacteria	100,000,000	1,000,000
Denitrifying bacteria	12,650,000	140,000
Azotobacter	1,000	1,000

organisms. The evidence derived from root studies of a wide variety of crop plants such as wheat, oats, barley, corn, timothy, mangels, flax, alfalfa, red clover, strawberries, and potatoes shows a distinct increase of bacteria requiring amino acids for optimum growth. On the roots of some plants from 50 to 60 per cent of the bacteria isolated are in this group, whereas in the soil a short distance away from the root only 15 to 25 per cent of the isolates are in this category. This observation fits in well with the many reports of excretion of amino acids by plant roots. Another striking effect is the selective action of the root on organisms capable of liberating ammonia from organic nitrogenous compounds, the so-called ammonifiers. These bacteria may be of great importance in the nitrogen nutrition of the plant.

We have recently found that there is a preferential effect at the root surface on bacteria which are capable of rapid growth and metabolism. This was expected but only recently confirmed, since among microorganisms (as among higher forms of life) certain species grow and multiply more rapidly than others, and in the presence of an available supply of nutrients these species will outgrow the slower forms and establish themselves as the predominant types. This phenomenon is observable even on roots that are only a few days old. The greater metabolic activity of these species, with respect to amino acids, proteins, and sugars is of considerable significance, since the decomposition of these substances results in the production of organic and inorganic acids and of carbon dioxide. The dissolving capacity of these products and the complexing or chelating properties of some of the acidic compounds are of importance in the mineral nutrition of the plant.

Although most of the work in the bacteriology laboratories at Ottawa has been with bacteria, some studies have been conducted with fungi as well. Selection of certain groups and species of these organisms also occurs at the root surface but we do not have sufficient evidence to warrant generalization. Very little information is available on the selective effect of roots on groups or kinds of fungi capable of carrying out specific biochemical reactions. This is an avenue of research that warrants much more attention not only with regard to plant nutrition but also in connection with root-rot problems.

Phosphate-dissolving bacteria isolated from soil and from the rhizosphere of wheat. The clear area surrounding four of the colonies indicates phosphate solubilization; one colony shows no activity.

Even less work has been done with the economically important nematodes or eelworms, than with fungi, in relation to the rhizosphere effect. However, there is some evidence that certain species of nonparasitic nematodes are attracted to and multiply in the root zone. It is not known whether the attraction is due directly to the influence of the plant or indirectly to the effect of root excretions on bacteria or fungi which serve as a source of food for the nematodes.

As has been mentioned above, once the rhizosphere effect manifests itself as a result of root penetration of the soil, interactions among the organisms themselves come into play and complicate the picture enormously. All the phenomena associated with the struggle for survival become operative; certain organisms or groups of organisms inhibit others by producing antibiotics or simply by using up the available nutrients faster than the others; certain types synthesize organic nutrients (amino acids, sugars) and vitamins which are excreted, or liberated on death of the cells, and are rapidly consumed by other forms; some bacteria and actinomycetes attack and decompose fungi, and many types of protozoa feed and multiply on bacteria. These are only a few of the relationships that exist among soil microorganisms, and in the rhizosphere there is an intensification of these effects because of the larger number of organisms involved.

Mycorrhizal formations on the root of white pine; produced by specific fungi which abound in forest soil. (Courtesy V. Slankis, Forest Biology Laboratory, Maple, Ont.)

We have been considering up to this point the effect of the plant root on the numbers and types of microorganisms developing in its sphere of influence. The reverse effect, that is, the influence of the soil microflora on the plant is of course of paramount interest and importance. The liberation of plant nutrients by the decomposition of organic materials in the soil and by the solvent action of the carbon dioxide and acids produced is a well-recognized function of soil bacteria, actinomycetes, fungi, and other organisms. This process is intensified in the rhizosphere owing to the density and metabolic activity of its micropopulation. For example, ammonifying bacteria develop in large numbers in the rhizosphere. These organisms liberate ammonia-nitrogen, which is readily available to plants, from nitrogenous substances which are not easily utilizable. Phosphate-dissolving bacteria have also been studied in relation to the rhizosphere problem. We have found that the roots do not exert a selective effect on these organisms but, owing to the large numbers present on the root and because they show greater metabolic activity than their counterparts in the soil away from the root, their influence may be very great. Recent calculations have shown us that there are approximately 6 to 18 times as many bacteria capable of dissolving insoluble phosphate compounds in the rhizosphere as there are in the surrounding soil. We found also that the organisms from the rhizosphere were twice as active as those in the soil away from the root.

Synthesis of amino acids, sugars, vitamins, and plant hormones (auxins) also occurs in the rhizosphere and recent evidence obtained in our laboratory points to a significant increase in numbers of bacteria in the root zone capable of such activity. Unfortunately the function of many of these substances in terms of plant growth is not clear as yet although such compounds as the gibberellins produce startling effects.

Microbial activity in the root zone is not necessarily beneficial to the plant. Competition between microbes and plants for nutrients such as nitrogen, sulphur, phosphorus, and trace elements may be so intense that the plant may suffer. A case in point is the well-known condition of nitrogen starvation in plants growing in soil in which straw has been incorporated. Rhizosphere bacteria cause manganese deficiency by oxidizing soluble manganese compounds to substances that are not available to plants. Bacteria and fungi in the vicinity of the roots also affect plants adversely by producing toxic substances.

More direct effects of certain types of soil microorganisms on plants are also well known. These are perhaps special cases but are nevertheless rhizosphere phenomena. The most striking and most important agriculturally is the fixation of atmospheric nitrogen by bacteria producing root nodules on legumes. Considering that it may add to soil annually as much as 200 pounds of nitrogen per acre, this microbial function is of the greatest significance. The penetration of certain woody and herbaceous plants by specific fungi, resulting in 'mycorrhizal' formation, is another special instance of a favorable effect produced by soil microbes on plants. Such structures, common among forest trees, are considered to be of considerable importance in reforestation projects. Again not all such associations are beneficial. Root-invading parasitic fungi or nematodes are also attracted to the root and cause a great deal of trouble. Many attempts have been made to overcome this by soil treatments designed to stimulate forms that are antagonistic to these pathogens—a form of biological control by creating a microbiological environment that is inimical to them. Thus control of strawberry root rot, potato scab, and cotton root rot may be achieved by treatment of soil with certain organic materials such as soybean or manures. Although these substances may affect the pathogens directly, their effect may also be indirect by stimulating a microflora that is antagonistic to the root pathogens. Evidence for the latter phenomenon is already at hand. Much emphasis has been placed by Russian workers on the benefits of seed inoculation with various organisms, some designed to stimulate plant growth and others to protect plants from root pathogens through antagonistic effects. Though the results have not been startling, a search for more active and more effective organisms should be continued.

It is evident that the rhizosphere is a unique zone, exerting on many soil organisms a powerful influence which varies with type, variety, age, and vigor of the plant and with type, treatment, and moisture content of the soil in which it grows. Increased knowledge of the specific reactions in this region may provide a basis for a better understanding of various phenomena related to plant feeding and growth, cropping systems, and root disease control. Such problems open up fields of investigation that are of great interest both academically and practically.

Swathing Studies . . . *from p. 7*

until the end of the test. The kernel moisture content of the standing grain decreased steadily as maturity advanced. Grain in the swath dried at a faster rate. The apparent stage of uniformity of maturity of standing grain again appeared at a kernel moisture content of 19 per cent. This was only one or two days in advance of the stage of maturity recognized as suitable for straight combining. Hence if a farmer waits to begin swathing until his grain reaches 19 per cent moisture content he might as well wait another day or two and straight combine thus saving an operation. In order to get all the advantages from swathing it should be done a week or more earlier.

Data collected over a period of years show that, on the average, not more than twenty days of suitable weather for straight combining can be expected. Thus, the advantage of gaining a week or so during the harvest season is particularly significant to farmers in the Prairie Provinces. The early harvest conditions, during which time the grain can be swathed, are usually more favorable than those that occur later in the season.

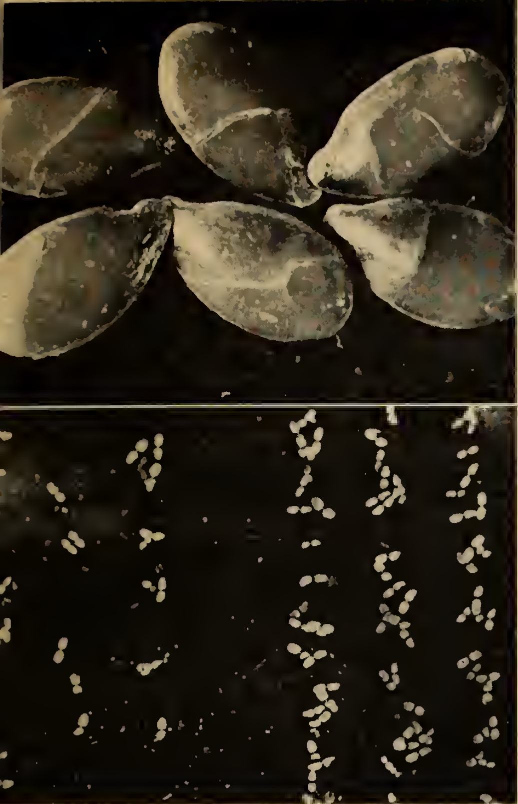
History of Swathing

Uniformity of ripeness and a low enough kernel moisture content to assure safe storage are the two controlling factors for a successful harvest by straight combining. The risk of losses from natural hazards and mechanical operation is increased as the maturity of grain advances.

The windrower, or swather, and the pick-up attachment for the combine were introduced to reduce these losses. In spite of the success of this operation the tendency to wait for uniformity of ripeness persisted and the advantage of early cutting became obscured. The short crops during the drought period of the depression years, caused a re-introduction of the swather with its associated implements, the header, and header barge. A third appearance of the swather, came as an effort to combat the sawfly problem. Since that time, swathing has become an accepted harvesting practice in Western Canada.

Seed Treatment of Cereals and Flax in Canada

J. E. Machacek



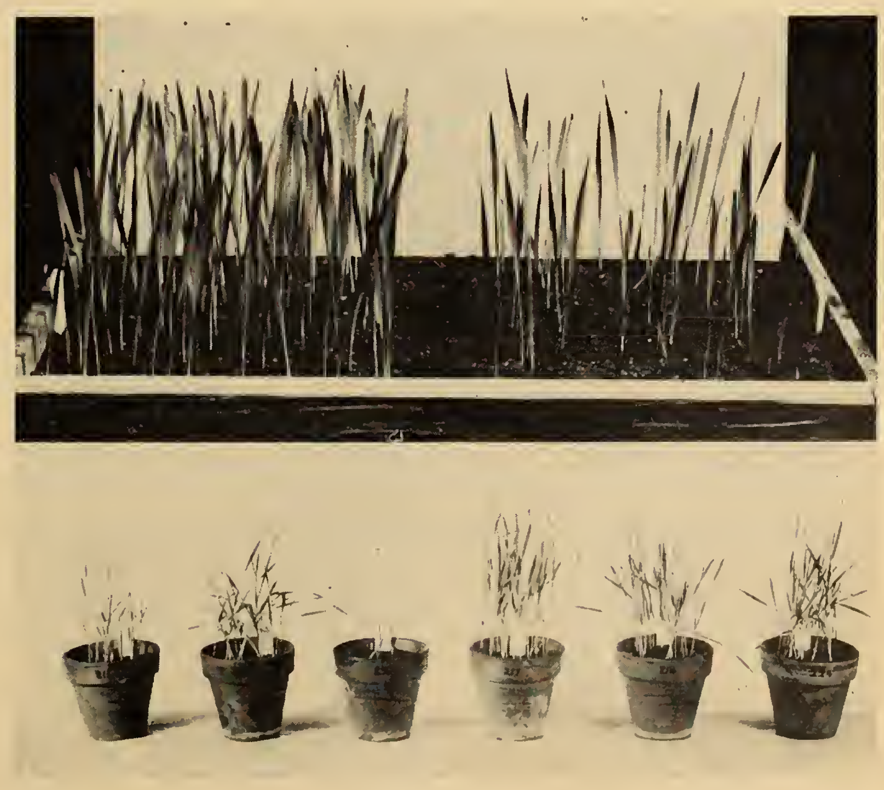
Seed rot of flax. Injured seeds (top) magnified to show cracks in seed coats. Lower photo shows seed germination improved by protective seed treatment (seedlings from treated seed above).

At the Canada Department of Agriculture Laboratory at Winnipeg, Man., one of our functions is to investigate the control of seed-borne diseases by seed treatment. The results of our work are used as a basis both for recommendations to farmers and for the registration of new seed-treatment products. In the studies, stations in other provinces of Canada and at various points in the United States co-operate.

The main purposes of seed treatment are : (1) to destroy the microorganisms which after overwintering on the seed may attack the subsequent crop, (2) to destroy the microorganisms that infect the seed during its development and in consequence cause disease in the new crop, and (3) to protect the seed from attack by soil-inhabiting microorganisms and insect pests.

We recommend that all seed grain be treated with one of the officially acceptable seed-treatment products before it is sown, unless a laboratory examination has shown that the seed is virtually free from disease and threshing injury. Wheat and rye seed, although generally free from surface-borne smut, sustains sufficient injury to the seed coat during threshing to make treatment worth while. Oat and barley seed should be treated because almost all of it carries a heavy load of spores of the surface-borne smuts attacking these crops. Flax seed warrants treatment because a high percentage of the seeds become cracked during threshing and, unless given a protective treatment, the cracked seeds may rot in the soil.

A wide variety of chemicals are used for seed treatment. Copper sulphate and formaldehyde, once popular, are no longer recommended because of their tendency to lower seed germination, especially in wheat injured by threshing. Modern fungicidal seed-treatment products fall into



Seedling blight of wheat. *Upper:* Germination of seed infested by a seedling-blight fungus (treated seed on the left). *Lower:* Some strains of the seedling-blight fungus are more virulent than others but all are controllable by seed treatment.

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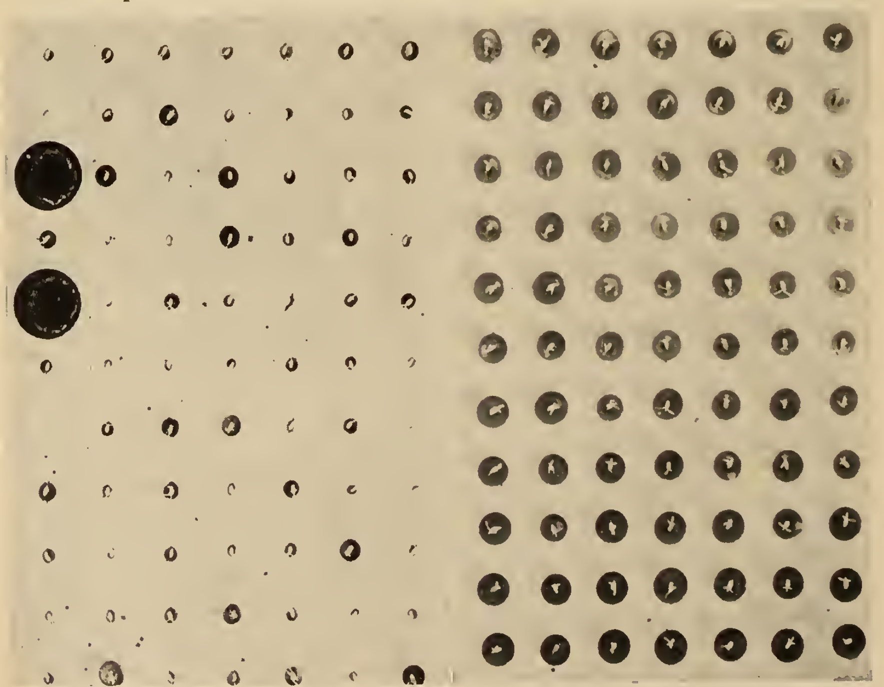
two classes: those that contain organic mercury compounds and those that do not. The active ingredients in the first class are generally one or more of the following: ethyl mercury acetate, methoxyethyl mercury acetate, ethyl mercury chloride, phenyl mercury acetate, phenyl mercury formamide, phenyl mercury urea, and methyl mercury dicyan diamide. Products with these ingredients are used for treating seed of cereal crops to control smut and seedling blight, and for treating flax seed to control seed rot. Products in the second class contain chloranil, captan, thiram, or hexachlorobenzene. Products containing any of the first three of the latter ingredients are used for the treatment of vegetable seeds to control "damping off". The fourth ingredient is very effective against bunt of wheat. Some seed-treatment products carry an insecticide to control wireworms, usually lindane, aldrin, or heptachlor.

Seed treatment products used in Canada are manufactured in the form of nonwettable, wettable, or soluble powders, or as "ready-to-use" or concentrated liquids. Some of the nonwettable powders contain a small quantity of oil to reduce their dustiness. Wettable powders contain detergents which, after the addition of water, permit their use in the form of a thin soup (slurry). The solutions made from soluble powders and the liquids are applied in small amounts only, so that the treated seed dries quickly. This variation in physical state reflects the evolution in products and their application in the search for a better seed treatment. Fifty years ago, the seed was generally dipped or soaked in a comparatively weak solution of a fungicide. This method of treatment was effective but the treated seed took a long time to dry, a serious defect when a large quantity of seed had to be treated, as wet seed tends to sprout. The advent of powder treatments removed the problem of drying the grain but introduced the new problem of flying, sometimes poisonous, dust. This flying dust is not a serious hazard to health when only a small quantity of grain is treated, but when it is treated on a custom basis, the necessary prolonged exposure to flying dust is not only objectionable but may be dangerous to the operator of the treating plant. The addition of a small quantity of oil to the powder reduced its dustiness to a considerable degree, but not completely, and so fungicidal slurries and concentrated liquids were developed. Products used as slurries or liquids are, however, difficult to mix uniformly with the seed unless fairly elaborate treating machines are used. Hence, farmers tend to use powder products, and custom plant operators slurries or concentrated liquids.



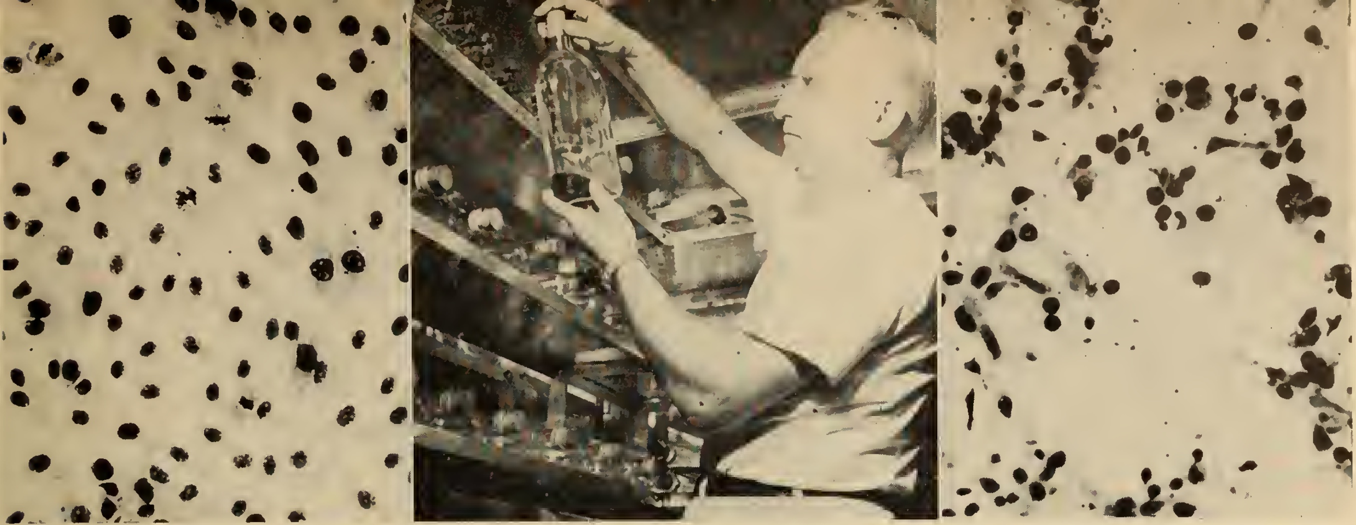
A test of uniformity of seed treatment. The size of the dark circles is directly related to the amount of fungicide carried by individual seeds. Treatment (left) not uniform; treatment uniform (right).

The evolution of seed-treating equipment paralleled the evolution of seed-treatment products. The introduction of powdered products has necessitated the use of some device with a closed chamber in which predetermined quantities of powder and seed could be mixed. Such a device may be home-made when the quantity of seed to be treated is small, otherwise a large-capacity machine, which in addition to a mixing chamber has a dispensing part to meter out seed and powder, is necessary. Slurries and concentrated liquids require both an accurate dispensing part and an efficient mixing chamber in which seed and fungicide are quickly and thoroughly mixed, otherwise irregular treatment results.

In recent years, many farmers have used motor driven, auger-type grain loaders as a part of their seed-treating equipment. Such loaders function fairly well when used as a mixing chamber for grain treated with fungicides in powder form but tend to be inadequate for liquids. Their use for mixing or conveying treated grain is not encouraged because of the danger of contaminating feed grain or food. In recent years, also, there has been a considerable increase in the number of custom grain cleaning and treating plants, especially in Ontario.

A number of problems connected with seed treatment still await solution. The most important of these is probably "mammalian toxicity". All of the "all-purpose" products on the market in Canada today contain mercury and are poisonous, while the mercury-free and comparatively safe products have limited use. So far, combinations of non-mercury organic compounds have failed to give satisfactory results. The danger of poisoning from seed-treatment products has been

(Concluded on page 14)



Technician (center) examining flasks of tissue culture in a walk-in Incubator; normal bovine embryonic skin tissue culture (left) magnified 180X, and bovine embryonic skin tissue culture (right) showing the destructive effect of the virus of coital exanthema of cattle (10X mag.).

TISSUE CULTURE

New Tool in Animal Disease Studies

Andrew S. Greig

MANY VIRUSES that cause disease in animals fail to grow or survive in any species but the one they normally infect. This fact imposes obvious limitations on the study of viruses, particularly of the viruses that infect human organisms or valuable domestic species of animals such as cattle. In recent years a new tool, tissue culture, has greatly broadened the virologist's scope.

Tissue culture is the culture or growth of animal cells outside the animal body. In one method, portions of tissue are removed from a freshly slaughtered animal and treated with an enzyme preparation which breaks the tissue down into its component cells. The free cells are then placed in a nutrient fluid in tubes or flasks where, after settling and adhering to the glass wall underlying the fluid, they proceed to grow and multiply. After a few days there is usually sufficient growth to form a complete sheet of cells attached to the glass, termed a "monolayer culture". Such primary cultures can usually be kept alive and healthy for two or three weeks or longer, provided the nutrient fluid is changed often enough to supply fresh food stuffs and remove waste products. Certain strains of cells have been kept alive and growing indefinitely through repeated transfer of actively growing cells.

Although the basic methods employed date back to 1885, the advent of the antibiotic substances reduced the danger of bacterial contamination and greatly extended the practical application of tissue culture. A wide variety of tissues derived from a large number of different animal species have been successfully grown. Although kidney tissue is most commonly used, skin, liver, heart, white blood cells, and even tumor cells have been employed.

When a suspension of virus is placed in contact with a monolayer of susceptible cells, the end result may be destruction or death of the culture cells. This destructive or cytopathic effect may serve as a useful indication of the presence of virus in suspected material. Alternatively, it may serve to detect antibodies to a particular virus in the serum of a convalescent or recovered animal. The serum is mixed with a suitable amount of the virus and the serum-virus mixture used to inoculate tissue cultures. If antibodies are present, the virus will be neutralized and no destruction of cells will occur. If there are no antibodies in the animal's serum, then the virus will proceed to infect and destroy the cells of the tissue culture.

Virus grown in tissue culture may also be employed for the manufacture of vaccines, and indeed the principal vaccines used to control foot-and-mouth disease in Europe are made with cultures of bovine tongue epithelium. Tissue cultures are also applied in sorting through countless drugs for their killing effect on viruses, so that eventually some may be found that will be as effective against viruses as the antibiotics are against bacteria.

At the Animal Diseases Research Institute, Hull, Que., we are making use of tissue culture in studies on several virus diseases of animals. Although we have employed primary tissue cultures effectively in several studies, we are chiefly interested in establishing strains of cells in continuous culture from several animal species. So far two lines of bovine embryonic cells have been developed, one derived from bovine embryonic skin and the other from bovine embryonic kidney. Each of these cell strains has been under continuous cultivation for almost 18 months. These two strains of bovine cells have been sent on request to other laboratories in Canada, the United States, and Europe. We have also established a strain of embryonic kidney cells from a pig foetus, and this strain has been cultivated for over ten months. In addition, work is in progress on the adaptation to *in vitro* growth of cells from the kidneys and skin of rabbit and sheep embryos. The bovine cell strains have been shown capable of supporting growth of the viruses of infectious bovine rhinotracheitis, coital exanthema, and

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vesicular stomatitis. Bovine rhinotracheitis, a mild upper respiratory disease of cattle, has been linked to some cases of "shipping fever", and is one of the group of mucosal diseases. So far it has not been reported in Canada, but since the virus is easily grown in tissue culture, this method can be used to detect its presence in tissues of any suspicious cases submitted for diagnosis. Coital exanthema is a disease that causes an acute inflammation of the genital mucous membranes of cattle. It is caused by a virus that was first grown in tissue culture at the A.D.R.I. from specimens taken from infected Ontario cattle. The possible relationship of this disease to infertility in cattle is being investigated. Vesicular stomatitis virus produces a condition that greatly resembles foot-and-mouth disease. The virus is easily grown in embryonated eggs and in tissue culture.

Author inoculating a tissue culture flask with infectious material.

Pig kidney cells are employed in studies on porcine atrophic rhinitis and an intestinal infection of pigs recently recognized in Ontario. This latter infection, frequently referred to as "vomiting and wasting disease of pigs", resembles a condition called "transmissible gastro-enteritis of swine" in the United States.

When the sheep and rabbit cell lines are established we hope to have available a wide enough variety of cells to grow most viruses that affect animals.

Tissue culture, although relatively new in the field of veterinary research, has already contributed a good deal to our knowledge of the viruses that infect animals. It is expected that it will continue to do so and will take its place beside the embryonated egg and the laboratory animal as an important tool in the fight to keep our livestock healthy.

Seed Treatment of Cereals and Flax in Canada . . . *from p. 12*

reduced somewhat by the fact that most of them now carry a dye which makes it possible to identify the treated grain, but even so mixtures of treated and untreated grains are difficult to detect. Another problem is the toxicity of some seed-treatment products to seed. A large dose is usually harmful, but in some instances a short exposure to only a small dose may seriously reduce seed germination. Copper sulphate, formaldehyde, organic-mercury compounds, and lindane under certain conditions, can do harm to most of the small grains grown in Canada. In addition, oat germination may be harmed by aldrin, and flax germination by chloranil.

A number of secondary problems arise from the physical state of the seed-treatment product used. Powders, for instance, vary greatly in volume per unit weight, and unless the treating machine is adjusted to take care of such variation, the use of light powders results in under-treatment, and the use of heavy powders in over-treatment. The error arises from the fact that the dosage is usually expressed by weight (ounces per bushel) although the machine dispenses the product by volume. While reduction in particle size by air-micronization increases efficiency of the product, the benefit may be lost by under-dosage unless allowance is made for its increased bulk. With liquid seed treatment products, the nature of the solvent or carrier affects their efficiency. Water can be used if the air temperature is above the freezing point, otherwise some kind of "anti-freeze" is needed. The kind of anti-freeze and the air temperature at the time of treatment affects the viscosity of the carrier, and indirectly its rate of absorption by the seed and the completeness of seed coverage. Theoretically, products with a low viscosity should be used in cold weather, and those with a high viscosity in warm weather. Volatility of the product also affects the efficiency of seed treatment. Volatile products are generally good seed disinfectants but only temporary seed protectants. Non-volatile products are generally good protectants, and may be good disinfectants also if seed and product are well mixed.

The quality of pesticides sold in Canada and the attendant labelling and advertising are kept at a high standard by the administrators of the Pest Control Products Act.

Covered smut of oats (smutty plants on right). An important plant disease controllable by seed treatment.

Left: Co-author Scott recording data in study of methods of sucker control to improve cigarette tobacco quality and reduce labor requirements. *Above:* A new type of hauling rack designed and built at the Research Station, Harrow, Ont.

Rebirth of Burley Tobacco Production

R. J. Haslam AND *W. A. Scott*

FOLLOWING a sharp decline in the production of burley tobacco in southwestern Ontario, this crop is now making a real comeback. After World War II despite a steady increase in cigarette consumption, the popularity of the stronger types of tobacco declined in both the domestic and export trade. Team work between workers at the Research Station, Harrow, Ont., and the growers and processors has raised the gross revenue from burley tobacco from $535,000 in 1953 to more than $3.75 million in 1958.

The authors are with the Canada Agriculture Research Station, Harrow, Ont. R. J. Haslam is head of the Tobacco Section, W. A. Scott is tobacco research officer.

Gearing production of burley tobacco to the cigarette business appeared to offer the best prospects for the return of a thriving burley industry. To this end we began investigations at Harrow in 1951 on methods of producing a new type of cigarette burley tobacco. We also studied procedures for growing and handling, seedling production, crop adaptation, methods of fertilization, effective means of harvesting and curing, and handling techniques after curing.

Varieties that had previously yielded the best quality cigarette tobacco were used in new methods of growing and handling burley tobacco. A new variety, Burley 1, developed at the Tobacco Experiment Station, Greeneville, Tenn., and originated from a cross between Harrow Velvet and a Greeneville breeding line, was the most suitable variety, with Harrow Velvet as a second choice.

Our experiments at Harrow and further trials on a commercial basis by key burley tobacco growers in the district revealed four essential points for growing

Left: Experimental barns for testing different methods of curing tobacco. *Right:* Tobacco grading and marketing officials examining tobacco cured by artificial heat.

Co-author Haslam (right) examining tall-growing burley varieties particularly adapted for cigarette tobacco production.

TABLE I

Trend in Number of Growers, Average Planting Allotments, Planted Acreage and Production of Burley Tobacco in Ontario, 1949-58. (Courtesy of the Ontario Tobacco Marketing Association)

Year	Growers participating	Average allotment	Planted area	Total production
	No.	acres	acres	'000,000 lb.
1949	2,942	3.86	11,385	15.5
1950	2,690	1.52	4,652	5.7
1951	2,093	1.17	2,480	3.6
1952	1,546	.97	1,406	2.4
1953	1,373	.79	1,096	1.7
1954	1,821	1.69	3,122	4.5
1955	2,124	1.84	3,916	7.0
1956	2,044	2.13	4,513	7.0
1957	2,158	2.73	5,884	8.1
1958	2,328	2.93	7,018	11.8*

*Preliminary estimate

the best quality cigarette burley—earlier planting (as soon after mid May as possible), close planting, late and very high topping, and a minimum of suckering. Earlier planting improved quality and increased yields, and the crop ripened at an earlier date when more favorable weather prevailed for harvesting and curing. Close planting (18 inches or less in the row) produced mild-tasting, thin-bodied, fine-textured, bright burley grades. A minimum of suckering until close to harvest maintained a thin-bodied tobacco and prevented the nicotine content from going beyond the desired level for best quality cigarette burley. Delaying suckering also saved labor. With low-topped burley tobacco at least two sets of suckers had to be removed. Much of this drudgery and high cost is eliminated in the methods of suckering recommended for cigarette burley.

Sandy loam and gravelly loam soils produced the best quality cigarette burley, although fair quality tobacco was also grown on the richer loam soils, provided the land was properly drained and a correct distinction made in fertilizer analyses and rate of application. However, the end result in choice of soil and use of fertilizer depended almost entirely upon correct field practice in growing and handling the crop.

Cigarette burley is no more fragile to handle than ordinary burley, but it is more susceptible to crushing and bruising. New procedures in harvesting involve cutting and needling the tobacco plants onto sticks in one operation with the sticks stuck upright in the ground. This all but eliminated bruising of the leaves, allowed for more uniform wilting, and gave greater protection from sunburn. We designed and built a suitable hauling rack at the Research Station to carry a large load, with the tobacco hanging in a vertical position, rather than pressed down on a flat rack. This type of rack kept the more fragile leaves from becoming bruised and torn, and with it one man less was needed to hang the tobacco in the barn.

While weather conditions in southwestern Ontario are fairly satisfactory for curing cigarette burley, we have used heat at Harrow with excellent results in improving quality and increasing yields.

Growing cigarette burley is still a new venture for many growers, but during the past few years burley tobacco production has increased sharply. Acreage allotments have been increased, and more and more growers are participating each year.

Left: Labor force needling cigarette burley tobacco at harvest by the new standing stick method. *Right:* Examining tobacco seedlings in the greenhouse.

The Queen's Printer and Controller of Stationery, Ottawa, 1959