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Routine Investigation of Seeds for Their Health Condition in the Dutch Seed Testing Station at Wageningen

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Testing seeds for their condition of health is now performed in several laboratories in both the old and the new world. However, in different places the emphasis is laid on different crops and diseases and different methods are used, so that it is next to impossible to judge the present state of development of health testing and to compare results. This publication has been composed in order to give foreign workers at least some idea of the development of health testing in the Dutch station. The author is convinced that this will be of greater importance for progress in this field than might be the account of some special experiments or experiences.

Thanks to the activity of Dr. Doyer, who started with health investigation of seeds more than 30 years ago, the inspection of seed samples for disease is fairly well rounded off in Holland. The main body of it was published in Dr. Doyer's I. S. T. A. Manual of 1938.

In addition, the Booklet of Methods of our Institute, published in 1951, contains a list of those diseases and pests for which we may inspect the seeds in routine investigation. In practice, however, the work is restricted to far fewer crops and diseases, as the senders of the samples seldom or never ask for health investigation of many seed species in which important seed-borne pathogens may be present.

Let me explain this nearer.

Naturally the brunt of our attack has of old been directed on the main agricultural crops, for here vast issues are at stake and consequently the interest of both research workers and men of practice is great. For the field crops much scientific information was available, and this facilitated the working-out of routine methods of inspection for disease in these species.

In Holland, moreover, especially the cooperation with the Certification Service for Agricultural Seeds (the N. A. K.), of which the main office is situated in Wageningen, is very close. The N. A. K. in the course of the years has issued general prescriptions for its customers, that all the agri-

cultural seed lots for export of certain of the main species have to be investigated for their health condition. Consequently most of the samples at present received for this investigation are from agricultural crops.

This in its turn is the reason why in most of our experiments we still are concerned with agricultural seeds, their diseases, methods of investigation, and the possibilities of seed treatment. This development has as its consequence that for the main agricultural crops we are capable of providing the owner of the seed with information concerning several important infections of each seed species, whereas for horticultural crops in most instances the information necessarily must be restricted to a single disease.

The reader will understand from this short introduction that the present situation is the result of a historical development and consequently has certain defects, and that it is difficult to escape from a *circulus vitiosis* in which the vegetable and flower seeds are so strongly neglected.

At all events, the present situation is such that there are perhaps a thousand diseases and pests for which the seed transmission has been proved or is very probable according to the literature. This collection is, however, in the first place restricted by the limited range of crops and diseases that are important in Holland. In the second place it is restricted by the limitations of our own knowledge and experience The result of these two restrictions is published in our Booklet of Methods. In practice, however, there is still a further restriction by the limited interest of our customers. What is left is laid down in our unpublished lists of official sample investigations and results, of which in due time I shall say more, and which are moreover summarized in our printed Year Reports.

First I have to explain that the Dutch Station receives three major types of samples. Shortly after harvest, and at our urgent request, we receive by means of the N. A. K. series of samples of the more important crops, which are used for gathering general information concerning the qualities of the new harvest. On the information gained with these orientation samples certain general measures and rules are based — concerning what is permitted and what has to be discarded for sowing and exporting purposes, concerning seed treatment, etc. — which measures are taken by the Seed Testing Station in concert with other Institutions concerned.

After having obtained this necessary information the bulk of the commercial samples gradually arrives. The commercial samples certainly are a heavier strain on our methods, for here the results have to be more accurate than in relation to the orientation samples.

Among the commercial samples the main body needs health inspection in relation to prescriptions of the N. A. K., viz. those from agricultural seed lots intended for export. The results of the health investigation, however, are only exceptionally recorded on the orange certificate. In by far the majority of the cases they are only sent to the exporting firm and the N. A. K. This fact again eases the stress on our knowledge, for then it is hardly necessary to use methods which give absolute information. Methods that provide us with comparable results, of relative value only, are sufficient for this purpose.

In addition, a number of commercial samples are investigated in relation to the General Certificate of Health of the F. A. O. (Rome Convention 1951). Since two years we perform this technical investigation for the Dutch Plant Protection Service, and we do this only for samples of agricultural crops. The figures obtained by us are forwarded to the Plant Protection Service, that on the basis of our information issues the certificate — or refuses it. The actual investigation of samples of horticultural seeds for the General Certificate is still performed by the Plant Protection Service itself. For these samples it is highly desirable to have absolute methods of investigation available, and the same is true in relation to I. S. T. A. referee samples. As, however, we only recently started with concerning ourselves with the General Certificate of Health, and likewise only recently started with investigating referee samples for their state of health — and this expressly with an eye to our methods — we have of old been very satisfied with our relative methods and their modest results.

I have already indicated that the health investigation of seeds in Holland is burdened with a past; certain trends in its historical growth have interfered with logic so that its present fabric is not wholly justified. Therefore this publication can only be edifying to you and to me if you are very critical and do not hisitate to forward your criticisms.

Let me go on with giving a short review of what we have actually done as to routine sample investigation in the year 1954/55. The total of samples investigated for their state of health in that year amounted to 4689 (if I have counted right). More than 600 of these were samples for our harvest orientation; nearly 3550 were of seed lots to be exported, of which about 550 were investigated with an eye to the General Certificate of Health. The rest were control samples from the district laboratories of the N. A. K., samples from research institutes, miscellaneous private samples, etc.

My lists, in which are jotted down the samples and the results, start with *wheat;* more than 1200 samples, of which about 100 for orientation.

This crop is investigated in a blotter test — 2 × 100 seeds in trays of about 10 × 25 cm, with a perforated bottom and a filter paper lining, covered with another filter paper, and stored for 3 days at 10° C and then 3 days at 20° C. After this time the trays are inspected with a binocular microscope for the presence of *Helminthosporium sativum* (the root rot fungus that is now so very important in North-America) and in addition for brown roots without *Helminthosporium* spores having been formed on the seed. This latter root rot is ascribed to *Fusarium* spp., without us worrying about the species concerned. Moreover 300 seeds are shaken with 30 cc alcohol, the alcohol is evaporated, the dry rest is taken up in 1½ cc water, and the resulting suspension is used for a spore counting under cover slip at 100 × magnification, in which the number of spores of *Tilletia caries* and *T. foetida* is recorded.

These procedures give reason for some remarks. In the first place: the blotter method is our main method, which we use for by far the most diseases of our program, and this we do not out of free choice but because we find ourselves in the midst of a gradual development. By now I have much experience with this method and consequently realize its merits and its shortcomings. I have no appreciable experience with alternative methods; I am often fascinated by their apparent merits, and on the other hand much impressed by their supposed defects. You will permit me in this publication only to pay attention to the things I know.

Then I must acknowledge that the blotter method for *Fusarium* spp. in cereals is a typical "relative" method. If a certain sample is germinated in blotters and inspected repeatedly, for instance after 4, 5 and 6 days, one may note percentages of, respectively, 35, 45 and 55% of brown roots. For that reason we have conditioned our test on 3 days 10° C + 3 days 20° C. The lower temperature is necessary in relation to afterripening, for we must have germination in this method in order to be able to observe the root rot. Still afterripening sometimes may spoil the fun. In general, however, we are well content with the results. In this relation I should like to refer to a field experiment conducted in last year's spring.

A number of spring wheat samples, which had displayed very different percentages of *Fusarium* in the blotter test but in other respects were rather much alike, was sown in the field, untreated and after mercurial treatment. The average *Fusarium* percentage of somewhat more than 34% agreed with

an average increase in emergence by the treatment of about 14%. There was a nice correlation between laboratory infection and field emergence: for the individual samples the difference in emergence between the treated and the untreated seed agreed fairly well with the sum of the percentage of heavy *Fusarium* in the blotter test plus ¼ of the percentage of slight *Fusarium* in this test. By the way, here you perceive one of the advantages of a germination test for health investigation, e. g. the distinction between slightly and severely infected seedlings.

After maturing and drying in the field the plant weight per row was determined, and it appeared that still the difference between treated and untreated was 15% on the average.

For the *Tilletia* determination we formerly used one hundred seeds per sample only. However, the results appeared to be very unreliable. Probably the reason for this is an uneven distribution of the bunt spores over the seeds, because they may be partly present as grit of the bunt kernels. This makes it possible that in one hundred seeds practically no infection is found, whereas in another hundred a large number of spores is counted. Consequently we raised the number of seeds per test to 300, but still judge it preferable in case of an appreciable infection being found to repeat the test with the double number of seeds.

The distinction between *Tilletia caries* and *T. foetida* is only of academic interest, but on the other hand is very easy.

Of *rye* we received some 80 samples, and most of these for our own orientation. Rye is only inspected for *Fusarium* spp. in the blotter test, for it does not pay to inspect here for *Helminthosporium sativum* although this fungus may occur.

Of *barley* 175 samples were investigated, the lesser half for orientation.

Here again the blotter test with 2 × 100 seeds is used. After 3 days 10° C + 3 days 20° C the seeds are inspected at 20 × magnification for *Helminthosporium sativum* and for *Helminthosporium gramineum/teres*. The former is mainly a root rotting fungus, before the war seldom seen in Dutch-grown barley but in recent years extremely prevalent in certain spring barley varieties. It is easy to distinguish between *H. sativum* and *H. gramineum/teres*, which cause leaf diseases, but distinguishing between the latter two is too difficult for routine work. Probably it also is not very important.

I have the impression that with the method indicated we do not catch all of the *Helminthosporium* infection. For hyphal infections of the seeds the duration of the test is probably sufficient to progress to formation of new

spores, but for superficial spore infection of the seeds its duration in all probability is not sufficient. So probably with our routine method the spore infection is neglected and here also the results are only of relative value.

Notwithstanding that, these results are rather satisfying. In 1955 we also carried out a field experiment with spring barley samples infected with *H. sativum*. The influence of the infection on the emergence was not great, but that could not be expected as also in blotters it only seldom caused seedling abnormality. The average improvement in emergence by fungicide treatment amounted to 10%, and the average improvement in plant weight after maturing to 18%. For the heaviest infected samples (infection up to a 100%), the increase in plant weight was even 55 to 65%.

It seems strange that such small differences in emergence percentage as those between the treated and non-treated wheat and barley samples of these experiments were not compensated by tillering. Probably the actual damage done by the infection is far greater than is expressed by the emergence figure because for restricting the latter practically only the severely infected seeds count, and moreover the favourable growing circumstances may at the same time be favourable for the later development of the root rot.

Furthermore, barley is examined for loose smut by the Canadian method of Russell, e. g. soaking the seeds in potassium hydroxide (10% at 15° C from 17 to 9 o'clock), gathering the scutella by means of wire sieves, dehydrating these with alcohol, clearing with lactophenol, and inspecting for the brownish mycelium at 10 or 20 × magnification. The disease percentages found are, however, scarcely worth all this trouble for the Dutch crop.

About 170 *oat* samples were received and investigated in the blotter test. *Fusarium* and *Helminthosporium sativum* are easily detected. In addition *H. avenae* may be present, but this species needs stimulation for spore formation. For this purpose Dr. Muskett had advised ultraviolet irradiation. First we had no success with a lamp as used for distinguishing between ryegrass species, but later we did have success with the germ-killing lamp of our inoculation room (5 min. at 15 cm distance). Yet one cannot expect that by this expedient all of the infection becomes visible; probably part of it will receive too much and part of it insufficient irradiation. At all events the method is a great improvement.

Investigation of referee samples, however, indicated the possibility that our present blotter test is too quickly finished to find all of this infection (3 days 10° C + 2 days 20° C, irradiation, another 2 days 20° C).

For *maize* (60 samples) seed-borne disease is of little consequence in our

cool summer climate. So we do not test this species for seed-borne diseases but make a cold-test in order to determine the resistance against the unfavourable sowing conditions that are normal in Holland. You will know that the cold-test was developed in the United States and is a kind of imitation of a field sowing. We use shallow trays for it, the same kind as is used for blotter tests, with less than 1 cm of loamy-sandy field soil underneath, and another thin layer on top of the seeds and in addition a covering moist blotter. 2 × 75 seeds per sample are used. The moisture content of the soil is kept rather low, at about 8% which is about 2/5 of the waterholding capacity. We do this in order to avoid a great error by fluctuating moisture conditions. At 50 to 60% of saturation this error would be far greater than at our level of about 40% of saturation. The stress of the unfavourable influences is laying on the temperature, which we have better in hand. After a week at 10° C the blotters are transferred to a 28° C germinator and then the test is finished in 3 or 4 days more. During the week at low temperature the seeds do not germinate but the omnipresent semi-parasitical soil organisms remain active so that for weak samples the damage may be great. We have had samples with a germinating capacity of more than 95%, and at the same time a cold-test emergence of the non-treated seed of less than 20%. Of course we also perform an additional cold-test after thiram treatment of the seed in case the sample was not taken from a treated seed lot.

Of late interest of Dutch seed firms in this method has slackened, for everybody is accustomed to sowing treated seed and for the treated seed the cold-test indeed is of less importance, as by the treatment the consequences of the weakness are at least partly eliminated.

It may be interesting to know that some years ago we received maize samples in the cob from a seed firm (for determining the quality in relation to payment of the contract grower by the firm). These samples were dried and shelled in the laboratory with small-scale equipment, and all of them showed a cold-test emergence of 90% or even higher. Later commercial seed samples from the same lots were received, of course processed by the firm with large-scale apparatus, and most of these appeared to have a cold-test performance of 20 to 50% (non-treated). In experiments with artificially injuring of the pericarp it can be nicely demonstrated that the closer to the plumule the scratches are made, the worse the cold-test result.

Of *peas* we had nearly 600 samples, of which a hundred for orientation and more than 400 in relation to export. All of these were of agricultural varieties including grey peas and marrow fats.

Peas are investigated in our laboratory in blotters that are provided with hollows for the individual seeds, and 4 × 75 seeds per sample are used. After 4 to 5 days already the seedlings are inspected with the naked eye for symptoms caused by *Ascochyta* and *Mycosphaerella* (these two are separately noted although their distinction is difficult and of little practical significance. No attention is paid to the distinguishing of *A. pinodella),* in addition for *Stemphylium* which in peas as well as in beans may be rather general, moreover for weak rotting seeds (this, of course, is not a matter of disease but an indication of weakness of the sample), and also 50 seeds per tray are opened for detecting symptoms of manganese deficiency (marsh spot). For these diseases slight and severe attacks of the individual seedlings are separately noted although it is realized that for *Ascochyta* spp. a slight attack on an emerging seedling may do more damage in the field than a severe attack that prevents emergence of a single seedling.

Beans are not important in our health department, as they are not an important exporting crop in Holland. We investigated less than 30 samples of agricutural beans. For horticultural beans, and likewise peas, health investigation would be very desirable but interest in fact is restricted to determination of the picking-over percentage. Our routine investigation of this crop includes *Colletotrichum, Stemphylium,* weak rotting seeds, and marsh spot. A spore preparation may be necessary to make sure that the symptoms observed in the blotter test are caused by *Colletotrichum* and not by *Ascochyta* spp. The distinction is justified because *Colletotrichum* is a very active parasite, whereas the *Ascochyta* spp. mostly only become apparent in the decaying crop. Marsh spot is far less prevalent in beans than in peas (the deficiency is very important especially in marrow fat peas). Moreover the seed weevil *Acanthoscelides* now has adapted itself more or less to our climate and is sometimes observed in Dutch-grown beans.

Vicia beans are not interesting to us, and in 1954/55 we only investigated some 20 samples. *Bruchus* weevils are often present; weak rotting seeds may reach considerable percentages; in addition marsh spot may be present, but seldom as a cause of abnormality. *Ascochyta* and other fungal diseases are very seldom observed.

Lupins (only 8 samples) also are not interesting. Most conspicuous are the weak rotting seeds, sometimes covered with *Botrytis* or other fungi. True parasites are not seen.

Fibre flax is the most important seed species in the health department. In 1954/55 we investigated more than 1900 samples, of which about 150 for

orientation and more than 1500 for export. This investigation again is performed in a blotter test, with 4 × 100 seeds per sample. For the bulk of the samples it is finished after 6 days, with only visual inspection for symptoms of *Botrytis cinerea* and *Alternaria* spp. *Botrytis* may be very prevalent in Dutch seed and very injurious in the field. Insignificant *Alternaria* spots were generally observed in the 1954 crop. Experiences with referee samples, however, made it probable that these were caused by *Alternaria lini*, which in the circumstances of the blotter test and on the rather weak seedlings acted as a weak parasite. Another disadvantage of the method is the difficulty of distinguishing between the *Botrytis* and the *Alternaria* symptoms in cases where no *Botrytis* mycelium or sporophores are visible. Yet this distinction is very necessary, for *Botrytis* is extremely important and *Alternaria* is not (except *A. linicola* for oil flax).

For certain samples — including those for countries in eastern Europe and those for countries which require the General Certificate of Health — the same trays are examined again after 12 days at room temperature, and then for *Fusarium* spp. (mostly *Fusarium avenaceum*), *Colletotrichum* (important in the 1954 crop), *Ascochyta (Phoma* sp.), and pasmo (up till now never observed in Dutch flax seed). It is clear now from soil tests and referee samples that *Ascochyta* is not fully evaluated with our method. When for instance we observe 1% of pycnide formation in the blotter test in fact a few percent of infection may be present. For good pycnide formation light seems to be necessary. Another restriction is that for *Polyspora* a separate method is needed. For detecting this pathogen one hundred seeds are put into water and inspected for the tiny spores in the swelling slime layer at 100 × magnification. I still omitted mentioning the occasional occurrence of *Sclerotinia sclerotia* and very seldom those of *Botrytis* among the seeds.

A few years ago I published that having determined the three factors germinating capacity, threshing injury and *Botrytis* infection we were capable of predicting an emergence figure. This certainly was not true for 1954. Samples of that year's crop often demonstrated a far lower greenhouse emergence than might be expected from the three factors mentioned. Additional weakness owing to extremely unfavourable ripening circumstances may have played a role here.

Radish and *Brassica* are germinated in blotters, which are perforated with a hundred holes to put the individual seeds into, in order to keep them from rolling. Radish tests are finished after 7 days, and then we often observe a far lower germination percentage than the official germinating capacity

indicates. This difference is caused by the removal in the official germination test of apparently normal seedlings which in some days more would have demonstrated abnormality owing to *Alternaria* infection or threshing injury. For the growing crop *Alternaria* certainly is not important. Cabbage samples are finished after 9 to 10 days at 20° C. Here *Phoma lingam* is most important, but I am afraid that with our routine method we do not observe all of it, as under the circumstances of the test (darkness) the fungus apparently is tardy with pycnide formation. Also it is not justified to consider those seedlings that show damping-off without symptoms of *Alternaria* infection as infected by *Phoma*. Here again we will either have to improve our method or we will have to shift to another method (nutrition agar; filter paper with prevention of germination by means of weed killer).

Beets, mainly sugar beets, are important for us only since we investigate agricultural seeds for the General Certificate. This started in 1954/55 with nearly 200 samples. Also here we use the blotter test, with 4 × 50 seeds, inspecting for pycnides after 12 days alternating 20/30° C. It is clear, however, that the circumstances of our test are not optimal for pycnide formation. Light appears to be an important influence. The subject is under investigation.

The group *Miscellaneous* is rather large since we are concerned with the General Certificate. Very important in it are Medicago species, clovers and ryegrasses. After inspecting the dry sample for sclerotia, mites, etc. the Legumes as well as the grasses are germinated on top of blotters. The *Legumes* are inspected after 10 days for *Phoma/Ascochyta* spp., *Colletotrichum*, *Stemphylium* and other infections. *Lolium* is inspected after 6 days for *Helminthosporium* (of which different types may be visible, apparently not only *H. siccans)* and *Fusarium*. In addition 100 ryegrass seeds are investigated in water for blind seed disease *(Gloeotinia temulenta)*, for which purpose each seed is put into its own drop of water, the glumes are teased off with needles, and the plate of glass with the drops is inspected at 100 × magnification.

By now I have finished the review of our routine sample testing. You may have observed how much of importance is missing: *Stemphylium* in carrots, *Colletotrichum* in spinach, *Septoria* in celery and parsley, etc. apparently are not interesting to commerce; several bacterial and virus diseases are likewise important but too difficult for us to determine.

The routine investigation of samples still leaves us time for research. In the preceding years this has mainly been dedicated to fungicides. However,

for the future we intend to shift the emphasis somewhat, in the first place to seed-borne diseases and the further development of routine methods for their detection, and in the second place to the study of seed weakness and the delevopment of routine methods for evaluating it. Both subjects are connected by that of fungicide treatment of seeds. The subject of seed weakness has only received passing attention in the preceding. It may be necessary to give a more coherent explanation here.

For certain seed species or varieties, and in other species for certain samples, there may exist a great difference between the germinating capacity (determined in the laboratory under favourable conditions) and the actual field performance (as a rule under rather adverse circumstances).

Radish seed is very strong. Even if it is severely injured by threshing one may still expect nearly the same germination percentage in the field as in pure sand or on top of blotters (provided that no seedlings are removed in a too early stage of development).

Maize in itself — at least the starch varieties — is also rather strong. However, threshing injury is very prevalent and after sowing the cracks in the pericarp serve as a gate of entrance to soil fungi, with often a great discrepancy between germinating capacity and field performance as its consequence. Also drying injury or injury by frosting of the maturing crop may play a role.

Flax is likewise a strong crop in normal years. Threshing injury may, however, severely increase the difference between germinating capacity and emergence in soil. In certain years there may be an additional weakness, probably caused by unfavourable circumstances of growing or storing, which still more increase this difference.

Peas and *beans*, especially the horticultural varieties, are as a rule very sensitive to soil circumstances. Probably high humidity during ripening and threshing is the cause of dead spots in the seed coat, in which after sowing the soil fungi may start their deteriorating activity. Also seed coat cracks caused by drying may be present.

Factors of weakness, that do not influence the results of classic testing methods in the laboratory, are more or less important in many other seed species, especially horticultural seeds. This subject is one of plant pathology and may be included in the conception of seed health, for this weakness is a matter of the equilibrium between seeds and soil fungi and of the way in which this balance is influenced by outward circumstances. Among these circumstances fungicide treatment of the seed is of outstanding practical

importance. Methods for determining this weakness, irrespective of its causes, will be very important for routine seed testing. Yet such a method at present is only available for maize. This certainly is a subject of enormous importance, that too long has been neglected and which deserves the attention of research workers and seed analysts alike.

Summary.

In the preceding an outline is given of the present state of health investigation of seeds in the Wageningen Seed Testing Station.

As a consequence of its gradual historical growth the work as a whole lacks a logical structure. It is mainly restricted to agricultural seeds, of which thousands of samples are investigated each year, first for the purpose of general orientation, afterwards in relation to export (partly with an I. S. T. A. certificate, partly with an F. A. O. General Certificate of Health).

As a rule detailed results of the investigation are not sent abroad.

For most diseases the method used has only relative value or otherwise serious shortcomings, and further development of methods consequently is an urgent necessity. Notwithstanding that, the work performed is of great practical value, as is illustrated by the results of some recent field experiments.

Research in the past few years has been dedicated mainly to the possibilities of fungicide treatment of seeds. In the future more attention will be given to the further development of the health investigation in its stricter sense (diseases, methods) as well as to the determination of seed weakness. Of the latter subject a short explanation is given.

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