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ASSESSMENT OF PHYTOSANITARY TREATMENTS ON DIFFERENT WHEAT SPECIES BY A GLOBAL METHOD, THE SO-CALLED THESIGRAPHY

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A b s t r a c t. An assessment test of phytosanitary treatments on 7 wheat species was carried out in Auvergne (France) through a global method - the thesigraphy. This analysis consists of making and decoding images of dendritic crystallization called biothesigrams. The images result from the rapid and controlled evaporation of an aqueous solution of $CuCl_2$ and a leaf extract. It is interpreted on the basis of three image parameters: texture, central figures and signs that inform about the level of denaturation and pollution of the substance under study. Those three parameters were evaluated according to eight leaf samples at the end of the vegetative cycle in wheat cultures. The count of thesigraphical spots is considered as an indicator of the intracellular occurrence of xenobiotic products. The morphology of the texture and central figures allows for the calculation of a semi-global thesigraphical index, indicating the plant vitality. The exponential increase of the spots within the 29 days after spreading pesticides expresses the gradual accumulation in the wheat leaves. Those products slowly disappear within the 21 days before harvesting. The semi-global thesigraphical index shows the parallelism between the vegetative cycle of the control wheat species and that of the treated ones.

K e y w o r d s: wheat, pesticides, global assessment method, thesigraphy

INTRODUCTION

Most of routine regulation controls use analytic methods to detect and measure the conventional components and the indesirable ones, such as pesticides. The global or systemic assessment methods are not very developed. Their impact for a diagnosis or primary orientation must be better documented.

For many years we have shown interest in the cupric or sensitive crystallization test which was proposed in 1935 by the German agronomist Pfeiffer [6,7], then improved by two physicians under the name of 'thesigraphy' (thesis: aggregate) first by Selawry [8] and then by von Hahn [5], and by Engquist [1] who studied the plant productions and modes of culture in Sweden.

The crystallization images result from the rapid and controlled evaporation of an aqueous solution of $CuCl_2$ containing an aliquote of the substance tested. The crystallization of thin layers from a heterogeneous and stationary solution yields dendritic or arborescent configurations which are part of fractal images.

In controlled experimental conditions of an air-conditioned cabinet (temperature, hygrometry, absence of dust and vibrations), the pure $CuCl_2$ solution crystallizes in uncoordinated, random and centrifugal aggregates, which corresponds to the zero-rate level in our system of reference (about 40,000 crystalline images).

In presence of an increasing amount of the substrate tested (wheat extract) the organization of rhombohedric shapes gradually becomes differentiated up to an optimal morphology of texture and figure specific to each sample.

The crystalline images are obtained by correctly choosing the substrate/$CuCl_2$ concentration rates to take the three following parameters into account:

- the texture with its radial striation and nucleation centres;
- the figures where the morphology changes from the initial vacuole to the final cross;
- the signs (spots) to evaluate the level of pollution of the wheat species tested.

The macroscopic examination of the two first parameters allowed Bige, Nash and Garel in 1986 to formulate a law of morphological involution that applies to the texture and central figures whose dynamics depends on the organization or disorganization of the substrate studied (Figs 1 and 2). The link between the initial vacuole to the final cross responds to the geometric entropy or degeneracy described by the last Fermat's theorem: $x^n + y^n = a^n$. Within the interval of $0<n<2$, this equation formally describes the various astroid forms, that we experimentally observed (Figs 1 and 2). Thus far, the decreasing change of n from 2 (the vacuole circle) to 0 (the cross) reduced to its cartesian coordinates over the winglet and the rosette ($n<1$) properly assigns the observed crystalline shapes emerging from the nucleation contour. Geometric entropy or degeneracy refers to the fact that the central shape involves degenerates or denatures into more and more relaxed and open shapes until its dissolving at the origin of the coordinates.

This method based on crystalline images is then used to directly evaluate the level of denaturation of a biological substance, whatever the cause and the level of pollution and/or physical stress including the impact of energetic electromagnetic radiations (UHF and Gamma). The thesigraphy seems to be recommendable as a global assessment tool of food quality (see review [8]).

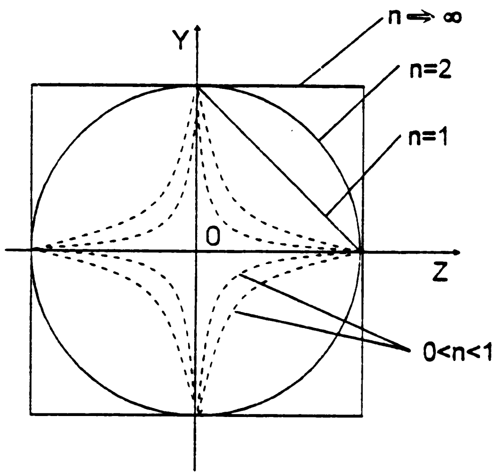


Fig. 2. Curve family originated from the Fermat's last theorem: $x^n + y^n = a^n$ (n functional, a fixed), which describes biocrystalline figures observed in Fig. 1. n<1 yields astroids which mimic winglet and rosette shapes.

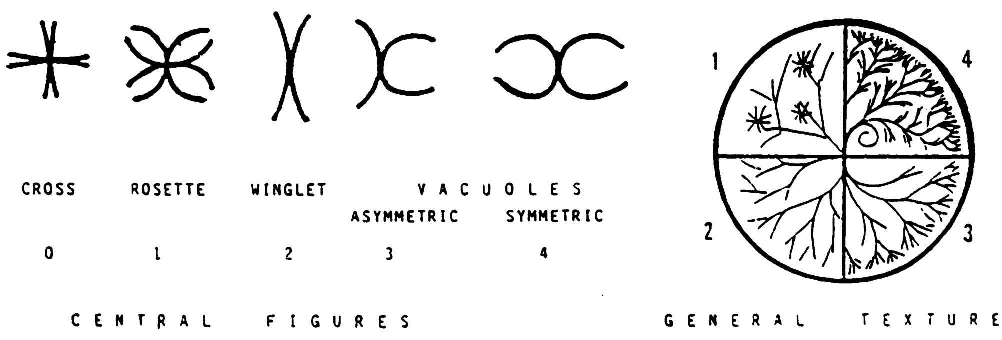


Fig. 1. Steps of morphological or geometric degeneracy of figures and texture from cupric dendrites. Step 4 is the native organized one for a biological substrate. Step 0 or 1 describes a highly denaturated, disorganized or degraded substances.

MATERIALS AND METHODS

Wheat culture conditions

The wheat species chosen for the experiment were cultivated in clayey and silt-laden soils (South Limagne in Auvergne) showing a low percentage of organic matter (2.27 %), a water pH of 7.2 and very high K and P contents (1.62 mg/100 g and 1.438 mg/kg, respectively).

The seven tender wheat species studied were all known for their high productivity (Briscard, Fidel, Marathon, Tarasque, Pernel and Garant).

In view of the control wheat species, on 21st April 1989 one part received 10 l/ha of TRIOTYL S (P.C.) active matter is based on M.C.P.P. + 2.4 M.C.P.A. + DICHLOROPROP and the other part received in addition to TRIOTYL S (P.C.) 3 l/ha of PRINTAZOL (active matter = 2.4 M.C. P.A. + 2.4D + PICHLORANE).

Subsequently, we shall then qualify the control species as pseudo-control species since the phytosanitary treatments were not harmless. On 30th May 1989 they were protected from the helicopter treatment as they were covered with plastic bags removed on the following day.

In the viewpoint of the treated wheat species they received through the aerial pesticide spreading 2 l/ha of TILT CT active matter = PROPIONAZOLE + CHLOROTALONIL and 0.3 l/ha of DECIS C.E. active matter: DELTAMETRINE on 30th May 1989.

Eight leaf samples were realized every 7-8 days from 1st June to 20th July 1989. After grinding and suspension in distilled water, the low-speed centrifuged supernatant (3.000 vpm/10 min) yields the mother substance stored at 4 °C during a few days if necessary for repeating the thesigraphical analysis.

Preparation of biothesigrams

After a preselection of the experimental conditions, we chose two discriminant concentrations. Each aqueous sample is spred 2-4 times on clear and fat-free glass plates fitted with removable rings.

The solution is evaporated during 12-15 hours in an air-conditioned cabinet (Striel 01 of 5 m^3 or Striel 02 of 90 l) at a temperature of 28 °C and hygrometry of 70 %. They must be at the most isolated from external electromagnetic fluctuations as well as from vibrations and dust in order not to disturb the dendritic crystallization process known to be unstable.

When the rings are removed, the images are stable at room temperature. They are stored until the thesigraphical analysis achieved either with an optical reader ZEISS fitted with magnification lens (6.5-17.5X) or with a videocamera CCD for adequate data processing.

Decoding

The biothesigrams are decoded according to two criteria:

- quantitative evaluation of the count of spots (signs);
- iconic parameters (texture and figures) that allows the calculation of a semi-global thesigraphical index.

Spots

For each biothesigram spots are recorded according to the size from 1 to 3 (for the biggest ones). The indicator 'spot' is then averaged for each homologous series of images. The so-called S indicator thus varies between 8 and 52. It equals 0 in the absence of spots. This range seems sufficient to assure that the phytosanitary products used are accumulated in the wheat leaves.

RESULTS AND DISCUSSION

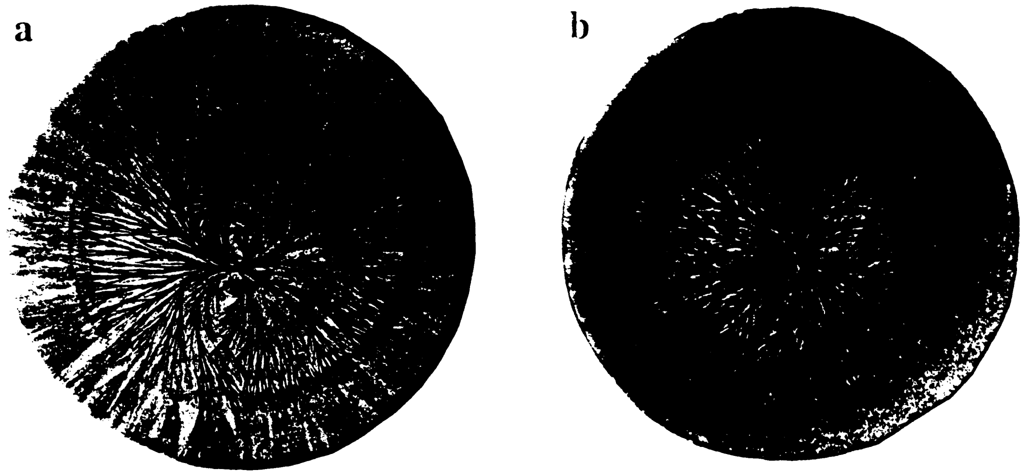
Fifty six leaf samples taken from 1st June to 20th July 1989 (harvesting) yielded a minimum of 450 crystalline images achieved in different experimental conditions, corresponding to two $CuCl_2$/substrate concentrations.

The images were indexed according to a visual analysis concerning the morphology of the three basic iconic parameters: texture, central figures and signs, i.e. spots. Thanks to specific software automatic decoding is currently studied (granted by the Ministry of Agriculture and Forests, Quality 2002).

The two first structural parameters respond to the law of geometric entropy or morphological involution that expresses the type of morphology according to the level of denaturation of the substance under study (Figs 1 and 2):

- The texture of any plant or animal biological substance of any simple or complex food product gradually changes from a dense morphology to a more relaxed one. We chose 4 coded levels to characterize the changes of morphology: dense (4), relaxed (3), lacunary (2), poor (1).
- The central figures produced by the convex crystalline stria from the centre(s) of germination or nucleation are characterized by a more and more relaxed morphology and by the obvious discontinuity (rosette phase) as well. We chose five typical and easily spotted coded figures: symmetrical vacuole (4), asymmetrical vacuole (3), double wing (2), rosette (1), cross (0).
- The spots which are due to the occurrence of foreign metabolites are counted and classified according to their size (see Methods).

The example of Fig. 3a explains the decoding protocol used for the calculation of two indexes: the semi-global index taking the morphology of both texture and figures into consideration and the spot index. The biothesigram of a young wheat leaf grown in a clayey and silt-laden parcel without any farming



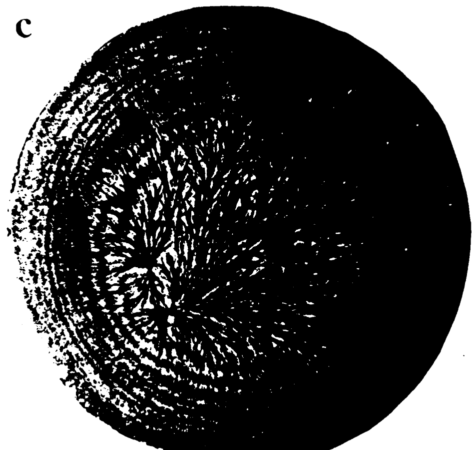


Fig. 3. Biothesigrams of: a young control wheat (a), pseudo-control wheat leaf after 16 days aerial treatment (b) and treated wheat leaf after 16 days (c). In February 1990 the leaf extract of wheat from Limagne (4-leaves phase) is co-crystallized with a cupric solution at controlled evaporation conditions. The frame is so dense, tight and well ramified. The central figure is an asymmetric vacuole, the indicator of a great vitality. The image is not polluted (absence of characteristic spots). Its semi-global index is high with 3.1/4.

intervention is characterized by a rather dense texture, an asymmetric vacuolary figure and the absence of spots. Its semi-global index is 3.1/4 and its spot index equals 0.

The biothesigram shown in Fig. 3b represents the morphology of a control wheat leaf sampled 16 days after aerial treatment. It comprises randomly distributed spots in the median and peripheral zones. Their number and density correspond to an S index of 18. The texture is more relaxed than that of the young leaf shown in Fig. 3b. The central figure is restricted to a small angular vacuole. Consequently, the semi-global index will be lower than the previous one: 2.7/4.

The biothesigram shown in Fig. 3c represents the morphology of a treated wheat leaf after 16 days of spreading. It clearly shows more spots than previous control or pseudo-control wheat. However, the texture remains well coordinated around the main stria with short needles. On the other hand, the central figure involuted, an incomplete rosette replaced the double vacuole. The semi-global index will be slightly lower than that ot the control, 2.4/4 and the S index will rise to.

All the crystalline images were indexed according to those examples. The representative value of the semi-global and S indexes and their variance give the two curves shown in Fig. 4a for control wheat and in Fig. 4b for treated wheat.

- For control wheat the curve showing the characteristic spots of the occurrence of pesticides in leaves was not much modified during the 35 days after treatment. In the end, a dramatic increase was noticed and then followed by a decrease.

- For treated wheat the increase in the number of spots is exponential during 29 days to reach a maximum. Then, they rapidly fall. So, during 3 to 4 weeks after aerial treatment, wheat accumulates xenobiotic components in the aerial parts which were the only ones tested. It is important to notice that biothesigrams from grains and different flour do not show or only a few spots due to pesticides.

However, control wheat manifests much slower

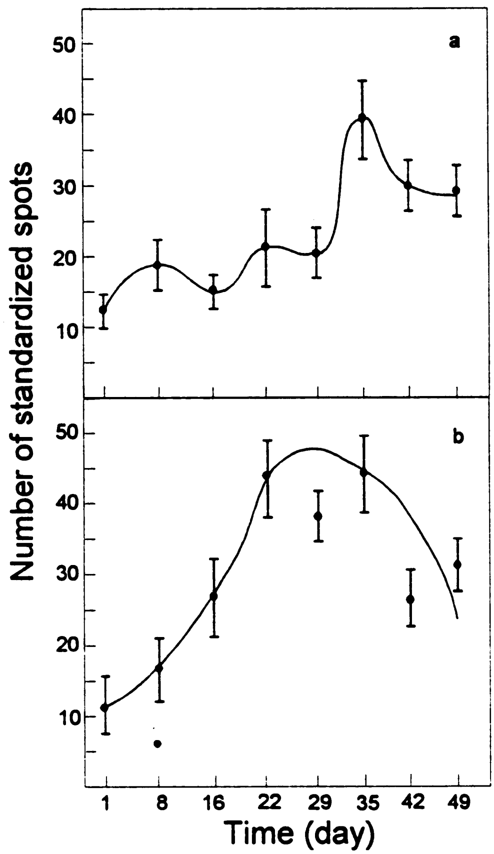


Fig. 4. Comparative patterns of the number of standardized spots for 7 wheat species. Pseudo-control leaves (a), treated leaves (b).

accumulation kinetics - about 4 times slower. However, after 35 days it is like treated wheat. Indeed, it appeared to us that we could not consider control wheat as a reliable control, but only as a pseudo-control. The islets protected from aerial spreading have an area of less than one square meter and are lost in the treated area which gradually diffuses according to the climatic events (edaphic pollution).

We further noticed the agreement between the initial values of the S index at the beginning of the experimentation (on 1st June) for the two groups of wheat. It correctly expresses the transforming effect on the whole parcel. This effect results from previous farming procedures, i.e., manuring (on 8th February 1989) and weeding with phytohormones of synthesis on 21st April 1989.

Finally, the curves shown in Fig. 5 compare the evolution of the semi-global thesigraphical indexes at the end of the vegetative cycle for the two types of wheat (control A and treated B one). Those two sigmoid curves express the decrease in vitality, normally associated to the maturation process. This vitality loss is the same for the two populations.

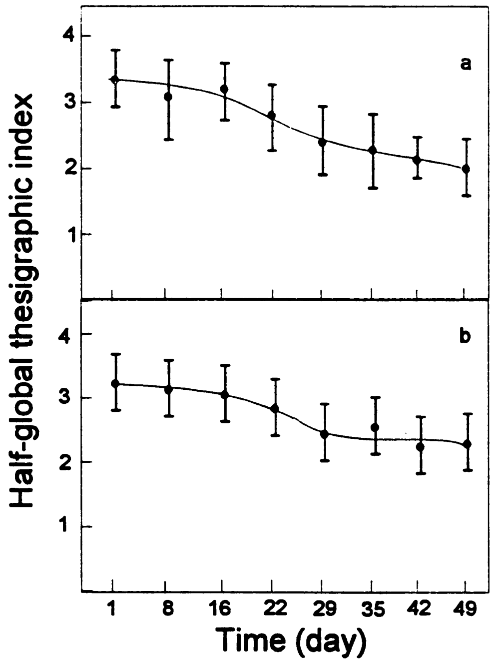


Fig. 5. Comparative semi-global thesigraphic index for pseudo-control (a) and treated wheat leaves (b) indicating the gradual loss of vitality at the end of the vegetative cycle 49 days before the crop.

Phytosanitary treatments at such doses used in Limagne do not alter the physiological evolution of wheat. The amounts spread there are twice less important than those spread for example in La Beauce, where yields are about 10 t/ha compared to 5.5 t/ha in Limagne.

The thesigraphical analysis of wheat confirm our previous observations about the direct identification of the 'spot' sign when non-natural products accumulate in the plant cells. We have already noticed clear differences with apple flesh obtained through the agrochemical farming method (16 treatments/year, the last one-one month before harvesting) producing many spots and through an ecological farming method in a slightly polluted environment, leading to very few spots.

On the other hand, in vitro studies carried out in our laboratory by Agns Mirmand in February 1992 on apple sections incubated during 24 h in solutions diluted with winter tender wheat weed-killers (CHLORTORIDE EL and CEPEDIC MP) showed a significant increase (more than 40 %) in thesigraphical spots in both Golden and Grany Smith species.

CONCLUSIONS

The thesigraphical analysis is an early detection method of xenobiotic substances. It can be used as an orientation test besides the conventional identification and dosage analyses. It is currently being validated to determine the sensitivity of this test for different families of phytosanitary components.

Recent observations on plants with a high vitality characterized by a high semi-global thesigraphical index (more than 3/4) tend to emphasize the hypothesis that those wild or cultivated plants rapidly metabolize the pesticides spread. Spots do not appear.

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