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PROPOSED INTERNATIONALLY STANDARDIZED METHODS FOR RACE IDENTIFICATION OF *Plasmopara halstedii*

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SUMMARY

Methods of determining races of sunflower downy mildew (*Plasmopara halstedii* (Farl.) Berl. & deToni) vary in both minor and major details, which may result in different interpretations. We propose the use of standardized methods and conditions of inoculation, incubation, and evaluation, which should make it possible to obtain essentially uniform results at any laboratory. We also suggest methods of surveying and collecting mildew samples to determine race distribution and prevalence. Sunflower lines which can be used to differentiate eight currently distinguishable mildew races, and the known or postulated resistance genes they carry, are listed.

Key words: Downy mildew, sunflower diseases, downy mildew races, race identification

INTRODUCTION

Race identification of *Plasmopara helstedii* and resistance evaluation of sunflower is practiced regularly by most sunflower research groups around the world. A whole seedling immersion inoculation techique is universally employed, but there are significant differences in procedures used at different laboratories. For example, inoculum concentrations used at different laboratories range from 20,000 to 200,000 zoosporangia per ml, inoculation times vary from 3 to 24 hours, and even the interpretation of susceptibility is not consistent (Champion, 1980, Cehen and Sackston, 1973, Piquemal, 1979; Vear, 1979; Viranyi and Bartha, 1981; Zimmer, 1974). In some cases, inoculation or evaluation procedures are not specified. The recent discoveries of new races in North America and new occurences of races on other continents (Gulya & Viranyi, 1991; Gulya et al., 1991; Tourvieille et al., 1988) make it vital that researchers studying mildew races use a common methodology to permit accurate race identification and allow meaningful comparisons between laboratories.

We propose a methodology which we believe to be as simple as possible and universally applicable, and which should consistently produce the same results in all laboratories. Most of the proposed methodology has been described previously, but many of the smaller details have not been specified. The method has proved satisfactory with

all races in the three laboratories of the authors. We have invited the help of scientists in several other countries to verify that the methods work satisfactorily for them as well. The specific materials we use, including manufacturers, are listed where necessary. They may not be available everywhere, but similar products from other manufacturers should work as well.

PROPOSED METHODOLOGY

1. **Downy mildew surveys and sample collection**. Downy mildew samples, even in the simplest survey, should be collected from every geographic/climatic region of the chosen study area. Additionally, every effort should be made to collect mildew from sunflower cultivars without any resistance genes. If that is not feasible, one should at least obtain information on what resistance genes are present in the cultivar being sampled. When collecting from fields with more than one cultivar (e.g. a breeding nursery), samples should be collected from as many different cultivars as is feasible. Surveys recommendations for the determination of disease incidence and severity are covered by Sackston (1978).

Since the occurrence of downy mildew in a given year or locality is sporadic and unpredictable, it is difficult to design a random survey and thus obtain unbiased information. In our experience, it may be more expedient to obtain information on areas of disease occurrence (from researchers, extension personnel, and growers enlisted beforehand) and then to sample these specific areas. The alternative of surveying vast areas with unknown mildew incidence can be very unproductive.

Individual infected plants, with sporulation, should be collected in labeled plastic bags, transported in a container with ice if possible, and the zoopsorangia used as quickly as possible. If plants bearing spores are not found, sporulation can usually be induced on detached leaves with mildew symptoms in the laboratory. Rinse the leaves under running tap water to remove dirt, place them in a closed container (petri dish or a moisture–tight box, with some wet paper toweling, and maintain at 16–18 ^{o}C in the dark. The leaves should produce fresh sporangia overnight. Viability of the spores can be checked with the aid of a fluorescence microscope and the vital stain fluoroscein diacetate, which will stain living sporangia yellow–green. A simpler method is to incubate the spore suspension used to inoculate seedlings for 3 hours at 15–20 ^{o}C and to examine for evidence of zoospore release.

2. **Standardized set of differential lines**. A proposed set of downy mildew differential lines is listed in Table 1, along with their known or postulated resistance genes. Inheritance studies have been done on some of these lines (Miller & Gulya, 1987; Miller & Gulya, 1991; Vear & Leclercq, 1971; Vrancaneau et al., 1981; Zimmer, 1974; Zimmer & Kinman, 1972). These lines have been tested at several laboratories and react consistently and uniformly with all races to date. The differentials are all inbred lines produced by government institutions and can be freely exchanged. Most are short season varieties (except HAR–4 and HAR–5) which will easily mature under most growing conditions and all are sufficiently self–compatible to produce good seed set under bags, with the possible exception of RHA 325. Small quantities of the lines may be obtained from the senior author after increase during the summer of 1991. Wherever possible these exact

lines should be used, even though lines of similar pedigree may give the same mildew reaction.

3. **Standardized evaluation procedures.**

a. **Seed preparation.** Surface sterilize seeds by immersing them in 20% household bleach (1% sodium hypochlorite) to which a small amount of detergent has been added to aid in wetting the seeds. Large–seeded types which tend to float in water can be submerged by placing a weighted object on the surface. Seeds should be treated for at least 1 minute; longer times up to 10 minutes will have no adverse effects. The seeds should then be thoroughly rinsed under running tap water, using a small screen sieve, until detergent bubbles are no longer present, when all traces of hypochlorite will have been removed.

b. **Germination.** The objective is to induce seeds to germinate as quickly and uniformly as possible, with a minimum of unusable seeds. Seeds may be placed on various types of absorbent paper, including paper toweling, seed germination blotters, and other similar type products. In our experience, the products which have been most satisfactory are machinery wiping towels (a non–woven, rayon fabric available from United Textile Co., San Lorenzo, CA or Chicopee, New Brunswich, NJ) or "Kim–Park" (Seedburo Equipment, Chicago, IL). It is important that the seeds be only one layer thick, and that they also be covered with wet paper or toweling to insure quick germination. In some laboratories the seeds are then rolled up in the double paper, producing what is commonly referred to as a "ragdoll." This method produces good germination, but is inconvenient because each ragdoll must be unrolled to check on germination. We find that placing seeds on paper on a perforated tray is the most convenient and efficient means of germination. The perforations allow drainage of excess water and having the seeds on a tray permits easy examination. The trays of seed are incubated in a germinator in the dark set at 22–24 °C.

The optimum seedling stage for maximum infection is when the radicle length is 5–20 mm and root hairs are just becoming evident. If the seed germinates well, we find that most genotypes used as differential lines will be optimum size in 2 to 3 days at 22–24°C.

c. **Inoculation.** Seedlings of the correct radicle length are selected. Seedlings with broken or brown root tips are avoided. Inoculum should preferably be fresh, produced during the previous night. If necessary, spores can be stored for several days in a refrigerator at 4°C. Allow the sporulated plants to air–dry for a few hours, cut them off at the soil line, and place them in plactic bags. No appreciable decrease in infectivity occurs with spores stored for 5 to 7 days. If the stored leaves start to decay, the effectiveness of the inoculum is significantly decreased due to contaminating bacteria and fungi.

Prepare a zoosporangial suspension in distilled or deionized water and use a hemacytometer to determine concentration. Concentrations between 20,000 and 50,000 zoosporangia/ml have consistently yielded 90 to 100% infection with the differential lines cited above; higher concentrations did not produce higher infection. Place seedlings of each differential line in a separate petri dish and fill with sufficient inoculum suspension to entirely cover the seedlings. Incubate, preferably in the dark, at 16 to 19 C for 3 to 5 hrs.

d. **Growing the seedlings**. Inoculated seedlings are planted in flats and grown for two weeks for syptoms of systemic infection to develop. After experimentation with field soils and many commercial "soil–less" mixtures, we have found that a sand/perlite mixture (2:3, v/v) produced the most satisfactory and consistent results. The sand need not be sterilized and either "concrete sand" or "fill sand" are satisfactory, whereas fine sand, commonly sold as "mason's sand" is not. Concrete sand specifications are listed as percent of the particles passing through a screen with the following size openings: 1.25 mm (1.1%), 2.5 (3.9%), 5 (15%), 8 (36%), 16 (66%), 32 (89%) and 63 (100%).

Rigid plastic flats (one example is the polypropylene "Dyna–flat" available in North America from Kadon Corp., Dayton, Ohio, U.S.) are filled with the sand/perlite mixture, thoroughly watered, and then rows of 20 holes are made with the aid of a "dibble board." Inoculated seedlings are placed, root down, in each hole. Twenty seedlings of each differential are planted, then the flats are watered again to settle the medium. Flats are kept in a greenhouse or growth chamber with temperature maintained between 17 to 24 C and a 12 to 16 hr. photoperiod, with light intensity averaging from 11,000 to 16,000 lux (maintained by supplemental light from 1000 watt high–pressure sodium lamps). Flats are watered daily; once per week they are fertilized with a commercial general purpose 20–20–20 liquid fertilizer (in the US, Peters, from R. Grace & Co., Fogelsville, PA, 18051).

e. **Evaluation and interpretation**. After 12–14 days, when the first true leaves are approximately 2 cm long, flats containing the inoculated seedlings are transferred to a chamber maintained at 16 C and 100% relative humidity. It is advisable to keep flats with different unknown mildew isolates physically separated to avoid spore mixtures. In some laboratories the flats are covered with clear plastic bags after misting the plants to insure isolation. Sixteen hrs. (usually 4 PM to 8 AM) in the dark is ample time to allow profuse sporulation. Susceptibility on the suggested differential lines is expressed by stunting, chlorosis, and sporulation on both cotyledon surfaces and frequently, but not always, on the first true leaves. The presence of profuse sporulation on the cotyledons and stunting of the plant, to include the leaves, are the main criteria for assessing susceptibility. Under the test conditions proposed we observe a minimum of damping–off due to downy mildew infection.

Occasionally we have observed 100% infection on several of the differentials and a lesser amount on one or more differentials. When we are certain of the seed purity of the differentials, we interpret this to be an expression of a race mixture. A race mixture can be verified by collecting sporangia from the line in question and used to inoculate seedlings of the same differential and a uniformly susceptible line. If the second (or third) inoculation produces 100% infection on both differentials, it proves that the original inoculum contained more than one race.

4. **Isolate preservation**. Scientists are encouraged to preserve any isolates representing a race not previously identified from their country. Long–term storage techniques, including the use of liquid nitrogen and ultra–low freezers, have both been successfully used (Gulya et al., 1988; Vear et al., 1987; Viranyi, 1985). The senior author and Dr. Sackston would also greatly appreciate samples of any new races. Both of us have official permits for importation of *P. halstedii* and have adequate containment facilities. Successful long–distance shipment of *P. halstedii* isolates depends on prompt delivery. We have had the best success when some type of "express mail" service is used. Samples

taking more than a week in transit generally are not viable when received. The easiest way to ship *P. halstedii* isolates is to send seedlings immediately after the inoculation procedure. Seedlings should be packaged inside a plastic petri dish or similar container filled with a damp water absorbent material such as cotton, vermiculite, or peat moss, and the container sealed with tape to prevent desiccation.

While individual researchers may use whatever race nomenclature system they prefer, it is encouraged that in international publications they refer to races by a virulence formula, based on the **P1** genes which a particular isolate can overcome (Sackston et al., 1990). The virulence formulas for the eight races identified to date are listed in Table 2. We propose that postulated new **P1** genes be referred to by alphabetic subscripts rather than numerical subscripts until genetic studies substantiate that the genes are indeed distinct from previously identified **P1** genes, as has been previously suggested for sunflower rust races (Ad Hoc Committee, 1988).

Table 1. Reaction of sunflower lines to known races of *Plasmopara halstedii*, causal agent of downy mildew.

Line	Postulated Mildew Genes	Reaction to Race						
		1	2	3	4	5	6	7
HA-300	–	S	S	S	S	S	S	S
RHA-266	$P1_1$	R	S	S	S	S	S	S
HIR-34	$P1_4$ + ?	R	R	S	S	S	R	R
DM-2	$P1_2$,$P1_5$	R	R	R	S	S	S	S
RHA-325	$P1_2$,$P1_B$	R	R	S	S	S	S	R
HA-61	$P1_2$,$P1_3$ + ?	R	R	S	S	S	R	R
RHA-274	$P1_2$,$P1_a$,$P1_b$	R	R	S	S	S	R	R
DM-4[a]	$P1_c$ + ?	R	R	R	R	S	R	S
DM-5[a]	$P1_d$ + ?	R	R	R	R	S	R	R
DM-6[a]	$P1_e$ + ?	R	R	R	S	S	R	R
HA-335	$P1_6$ + ?	R	R	R	R	R	R	R
HA-337	$P1_7$ + ?	R	R	R	R	R	R	R
RHA-340	$P1_8$ + ?	R	R	R	R	R	R	R
HA-R4	$P1_f$ + ?	R	R	R	R	R	R	R
HA-R5	$P1_g$ + ?	R	R	R	R	R	R	R

Seed of differentials DM-4, DM-5 and DM-6 is being increased during the summer of 1991 and will officially be released in the fall.

Table 2. Proposed nomenclature of *Plasmopara halstedii* races based on the P1 genes each race can overcome.

Old,chronogical designation	Proposed virulence Formula
Race 1	Race 0
Race 2	Race 1,5
Race 3	Race 1,2,3,4,b
Race 4	Race 1,2,3,4,5,a,b,e
Race 5	Race 1,2,3,4,5,a,b,c,d,e
Race 6	Race 1,2,5,b
Race 7	Race 1,2,5,c
Race 8	Race 1,2,3,4,a,b

DISCUSSION

Due to the recent resurgence of interest in sunflower downy mildew world–wide, an extended discussion of the research behind some of the above methodology may be in order, especially since most negative data are not published.

Survey procedures and sample collection. Conducting a survey to determine geographic distribution and frequency of races is hampered by two main factors. First, downy mildew occurrence is heavily influenced by environmental factors. Thus, the lack of diseased plants does not imply the absence of the pathogen. Second, due to the scarcity of published accounts of surveys on sunflower downy mildew and related fungi, there are relatively few guidelines on surveying and sampling procedures (Crute, 1987). I an attempt to counter the environmental effect on disease occurrence, collection of fungal propagules may give more accurate information on pathogen distribution. Two collection methods, which have not been documented with sunflower downy mildew, are soil sampling and collection of air–borne spores. In fields where downy mildew has occurred previously, bulked soil samples should contain oospores. Once the conditions for oospore germination are determined, it may be possible to determine races by planting the differentials directly into the soil samples. Collection of aerial spores is commonly practiced with rust and powdery mildews. *P. halstedii*, however, is not known to be widely wind–disseminated and the zoosporangia are short–lived and delicate, compared to rust urediospores. If collection techiques were perfected, however, this method might provide more accurate information on fungal distribution than by collecting spores from diseased plants.

In a recent study examining mildew distribution in the U.S. (Garcia and Gulya, 1991) almost half of the fields sampled had more than one mildew race present, and in some cases as many as five races were identified from an individual field. There were also several instances in which two races were identified from an individual plant. This demonstrates the need to limit samples to individual plants. When race mixtures do occur they can often be separated and purified by increase on specific differential lines, but in many cases the appropriate differentials have not yet been developed. Single zoosporangium isolates would be the only reliable means of obtaining a genetically pure isolate of a single race. Unfortunately, singe sporangium inoculation is very time consuming and our success rate is only about 15–20%, making its use impractical for large scale surveys.

Differential lines. The lines listed in Table 1 are all releases of the USDA Oilseed Project with the exception of HIR 34, produced by INRA in France. While all resistance genes identified to date are single, dominant genes, we feel it is more appropriate to use inbred lines rather than hybrids or open–pollinated varieties as differentials. The lines are genetically and phenotypically uniform and self fertile, so seed can be increased easily by self pollination. The first two authors are currently working on the development of additional differentials to include (1) a set of isogenic lines with susceptibility to only one race at a time and (2) a set in which individual isogenic lines are resistant to only one race.

There are other lines with pedigrees close to or identical to those listed in Table 1, and we realize that several alternate genotypes may be used in lieu of some of the primary differentials listed. However, we have encountered cases recently where two lines with

presumed identical resistance genes were able to differentiate between two isolates. Therefore, we propose that the lines in Table 1 be considered as the primary set of differentials. If additional genotypes are used, they may either confirm the outcome of the primary set, or possibly distinguish yet another race. The authors would welcome suggestions and/or seed of any genotypes which might be useful as mildew differentials.

Inoculation and "grow–out" procedures. There is considerable variation in the inoculation procedures reported in the literature, primarily with regard to inoculum concentration and the duration of inoculation. Our observations over the past several years have substantiated that 20,000 sporangia/ml for 3 hrs. is sufficient to induce close to 100% infection with any race on any of the appropriate susceptible differentials. Inoculum concentrations appreciably higher than 20,000 spores/ml often result in more seedlings damping–off, and thus can be counterproductive. Similarly, inoculation periods of 18–24 hrs. are more apt to result in damping–off, due to both downy mildew and contaminants in the inoculum.

Some conditions during the two–week grow–out period have a profound effect on symptom expression while others have only a negligible effect if any. The planting medium is a major factor, influencing the infection percentage, the severity of symptoms, and the convenience of the procedure. We have found a sand/perlite mixture to be highly satisfactory. Sand is essentially pathogen–free without the need for sterilization, drains readily and thus does not favor damping–off. It is relatively clean to work with and is fairly uniform world–wide, making it an easy medium to duplicate. The perlite is added primarily to decrease the weight, although it also improves aeration. Seedlings planted in soil, in contrast, may damp–off due to other fungi, necessitating some type of pathogen–free planting medium. Field soil is probably the least desirable medium, at least for attempts at standardization, because it is quite variable. Several commercial potting mixtures containing varying proportions of peat, vermiculite, perlite, sand, and in some cases, fertilizer, have been tested (Ljubich, 1989). Most were inferior to a sand/perlite mixture and at least one reduced infection by 75%, suggesting that a mycoparasite may have been present in the peat moss.

Nitrogen content has been demonstrated to have a profound effect on both disease incidence and symptom expression (Mohammed and Viranyi, 1983; Ljubich, 1989). In sand without any additional fertilizer, the symptoms on inoculated plants are primarily restricted to the cotyledons, a phenomenon referred to as "cotyledon–limited infection." At 100 ppm nitrate nitrogen, disease incidence is maximum and the symptoms are primarily systemic (Ljubich, 1989). At concentrations greater than 100 ppm, there is a substantial decrease in disease incidence. Ammonium nitrogen sources have a deleterious effect on mildew and should be avoided. If 'Peters' fertilizer is not available, a solution of sodium nitrate (8.5 mg/l to supply 100 ppm nitrate nitrogen) can be substituted.

Both light intensity and photoperiod have a minimal effect on disease incidence and symptom expression (Ljubich, 1989). We have observed no significant differences with photoperiods of 8, 10, 12 or 16 hrs. under greenhouse or growth chamber conditions. Similarly, light intensities in growth chambers ranging from 170 to 350 microEinsteins/m^2/sec did not appreciably affect disease incidence. Both infected and non–inoculated, healthy plants were shorter under the higher light intensities, but there was no effect on mildew symptom expression.

Contamination of flats and planting medium is of greatest concern when determining the race of unknown isolates. In our experience hard plastic flats are preferable to those made of wood or metal because plastic flats are easy to clean of planting medium and sunflower roots, do not rust or rot, and can be sterilized if desired by immersion in alcohol or hypochlorite solution, or by autoclaving. The sand/perlite mixture is relatively inexpensive and in our opinion it is preferable to discard the mixture after one use rather than attempt to sterilize it and run the risk of carryover oospores.

Evaluation and interpretation. Under the conditions described, all differentials react uniformly will all races tested to date. Resistant differential–race combinations have displayed no evidence of stunting, chlorosis or sporulation on any aboveground portion of the seedling. Susceptible differential–race combinations display stunting, chlorosis and sporulation on cotyledons and first true leaves, and only infrequently some damping–off. Frequently we observe situations in which the cotyledons have profuse sporulation but the first true leaves have none. If the seedlings are stunted and the size of the true leaves reduced, however, we still categorize these seedlings as susceptible. None of the differential lines proposed respond to any of the eight races with "twisted leaf syndrome" (Hoes, 1983).

When a differential has a low percentage of susceptible seedlings and other lines show close to 100%, we presume that the original inoculum was a mixture of two or more races. As little as two zoosporangia/ml can produce up to 10% infection (unpublished data, Gulya), but the amount of infection is dependent upon both the mildew isolate and the differential. With a standardized concentration of 20,000 zoosporangia/ml, a second race can be detected in proportions as low a 0.01%. It is easier to detect a small amount of a more virulent race in an admixture with a large amount of a less virulent race than the opposite combination. By collecting spores from partially infected differentials and reinoculating those same differentials we have successfully purified several race mixtures. Unfortunately the differentials currently available will not allow separation of all possible race combinations. The use of *P. halstedii* race–specific DNA probes with either the dot blot method or restriction fragment length polymorphism (RFLP) analysis may be an alternate and more precise means of race identification in the future (Borovkov et al., 1991).

CONCLUSION

We wish to emphasize two points. First, that we are attempting to standardize procedures for identifying races of downy mildew. We are proposing methods which we have found to be effective and relatively simple. We trust that colleagues elsewhere in the world will test them and inform us of any difficulties which they may have encountered under their conditions. Researchers are invited to share details of their methodology with us, especially where techniques other than the ones presented have produced satisfactory results. We hope that we can achieve agreement on a set of procedures to be presented at the International Sunflower Conference in Pisa in 1992.

Second, although standardization is essential to achieve comparable results in identifying races in different laboratories, it is not as critical for routine tests of reaction of sunflower germplasm to downy mildew in breeding programs, or for routine tests of

the effectiveness of various control practices. Every laboratory should continue to use the methods found to be most effective and convenient for such routine work.

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REFERENCES

Ad Hoc Committee. 1988. Report on naming system for sunflower rust races. Proc. XII Int. Sunflower Conf. (Novi Sad, Yugoslavia). pp. 250–255.

Borovkov, A. Y., I. G. Borovkova, B. A. Vick, P. E. McClean and T. J. Gulya. 1991. Discrimination of sunflower downy mildew, *Plasmopara halstedii*, races by DNA probes. Proc. National Sunflower Assoc. Research Workshop, Fargo, ND, USA. p. 1–2.

Champion, R. 1988. Sunflower: Official laboratory disease testing in France. Checking of varietal resistance to downy mildew (Plasmopara helianthi). Proc. XII Int. Sunflower Conf., (Novi Sad, Yugoslavia). 3p. handout.

Cohen, Y. and W. E. Sackston. 1973. Factors affecting infection of sunflowers by *Plasmopara halstedii*. Can. J. Bot. 51:15–22.

Crute, I. R. 1987. The geographic distribution and frequency of virulence determinants in *Bremia lactucae*: relationships between genetic control and host selection. Pages 192–212 In: Populations of plant pathogens. M. S. Wolfe and C. E. Caten, eds. Blackwell Sci. Pub., London.

Garcia, G. and T. J. Gulya. 1991. Sunflower downy mildew race distribution in North Dakota and Minnesota. Proc. National Sunflower Assoc. Research Workshop, Fargo, ND, USA. p. 3–5.

Gulya, T. J., R. R. Rojas and S. Maširević. 1988. Long–term storage of *Plasmopara halstedii* zoosporangia in liquid nitrogen without cryoprotectants. Proc. XII Int. Sunflower Conf. (Novi Sad, Yugoslavia). Vol. II, pp. 38–41.

Gulya, T. J., W. E. Sackston, F. Viranyi, S. Maširević, and K. Y. Rashid. 1991. New races of the sunflower downy mildew pathogen (*Plasmopara halstedii*) in Europe and North and South America J. Phytopathology: In Press.

Gulya, T. J. and F. Viranyi. 1991. Races of sunflower downy mildew in Hungary and comparison of Apron tolerance between U.S. and Hungarian isolates. Proc. National Sunflower Assoc. Research Workshop, Fargo, ND. pp. 6–7.

Hoes, J. A. 1983. Twisted leaf, a newly recognized syndrome of systemic sunflower downy mildew. Can. J. Plant Pathol. 5:206.

Ljubich, A. 1989. Sunflower downy mildew (*Plasmopara halstedii*) and environmental factors affecting seedling reaction to artificial infection. M.S. Thesis. North Dakota State University. 123pp.

Miler, J. F. and T. J. Gulya. 1987. Inheritance of resistance to race 3 downy mildew in sunflower. Crop Sci. 27:210–212.

Miler, J. F. and T. J. Gulya. 1991. Inheritance of resistance to race 4 downy mildew derived from interspecific crosses in sunflower. Crop Sci. 31:40–43.

Mohammed, S. A. and F. Viranyi. 1983. Effect of nitrogen supply on downy mildew development in sunflowers grown in perlit culture. Acta Phyopath. Acad. Sci. Hung. 18:281–290.

Mouzeyar, S., F. Vear, J. Philippon and D. Tourvieille. 1991. Downy mildew race distribution in France and a new hypothesis concerning resistance genetics. Proc. Nat. Sunflower Assoc. Research Workshop, Fargo, ND. pp. 8–11.

Piquemal, G. 1979. Screening sunflower seedlings for downy–mildew resistance. Sunflower Newsletter 3(2):9 10.

Sackston, W. E. 1978. Sunflower disease mapping in Europe and adjacent Mediterranean countries. Proc. VIII International Sunflower Conference. Minneapolis, MN. p. 7–29.

Sackston, W. E., T. J. Gulya and J. F. Miller. 1990. A proposed international system for designating races of *Plasmopara halstedii*. Plant Disease 74:721–723.

Tourvieille, D., R. Champion, F. Vear, S. Mouzeyar and J. Said. 1988. Une nouvelle race de mildiou en France. Identification, test et controle. CETIOM Inf. Tech. 104:3–7.

Vear, F. 1978. Reaction de certains genotypes de Tournesol resistants au Midiou (*Plasmopara helianthi*) au test de resistance sur plantule. Ann. Amelior. Plantes 28:327–332.

Vear, F. and P. Leclercq. 1971. Deux nouveaux genes de resistance au mildiou du tournesol. Ann. Amelior. Plant. 21:251–255.

Vear, F., D. Tourvieille and J. Philippon. 1987. Test de resistance au mildiou chez le tournesol: nouvelles techniques de conservation de l'inoculum. CETIOM Inf. Tech. 98:19–20.

Viranyi, F. 1985. A simple technique for long-term storage of *Plasmopara halstedii* zoosporangia at low temperatures. Trans. Brit. Mycol. Soc. 85:529–531.

Viranyi, F. and M. Bartha. 1981. Evaluation of sunflowers for the degree of resistance to downy mildew. Acta Phytopath. Acad. Sci. Hung. 16:265–267.

Vranceanu, A. V., N. Pirvu and F. M. Stoenscu. 1981. New sunflower downy mildew resistance genes and their management. HELIA 4:23–27.

Zimmer, D. E. 1974. Physiologic specialization between races of *Plasmopara halstedii* in America and Europe. Phytopathology 64:1465–1467.

Zimmer, D. E. and M. L. Kinman. 1972. Downy mildew resistance in cultivated sunflower and its inheritance. Crop Science 12:749–751.

METODOS ESTANDAR PROPUESTOS INTERNACIONALMENTE PARA IDENTIFICAR RAZAS DE *Plasmopara halstedii*

RESUMEN

Los métodos de determinación de razas de mildiu (*Plasmopara halstedii* far and de toni) varian en detalles mayores y menores entre laboratorios en diferentes paise, los cuales pueden a veces resultar en diferentes interpretaciones. Se proponen métodos estandar y condiciones de inoculación, incubación y evaluación, los cualesd deberian hacer posible la obtención de resultados uniformes en cualquier laboratorio. Se sugieren también métodos de inspección y colección de muestras de mildiu las cuales pueden ser utilizadas para diferenciar las ocho razas distinguibles actualmente. Y se enumeran los genes conocidos o postulados que llevan.

PROPOSITION D'UNE MÉTHODE STANDARD INTERNATIONNALE POUR L'IDENTIFICATION DES RACES DE *Plasmopara halstedii*

RÉSUMÉ:

Les méthodes de détermination de races de mildiou du tournesol (*Plasmopara halstedii* (Parl.) Berl. & De Toni) varient à la fois par des détails mineurs et majeurs selon les laboratoires et les pays, ce qui peut conduire parfois à une interprétation contradictoire des résultats. Nous proposons une méthode standard, des conditions d'inoculation, d'incubation et d'évaluation, qui pourraient permettre l'obtention de résultats uniformes dans n'importe quel laboratoire. Nous suggérons également l'utilisation de méthodes de conservation et de collecte d'échantillons afin de déterminer la distribution et l'importance relative des races.

Nous donnons aussi la liste des lignées de tournesol qui peuvent être utilisées pour différencier les huit races actuellement distinguables et les génes de résistance connus ou supposés portés par ces lignées.