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PROBLEMS WITH THE LEAF DISK IMMERSION (LDI) METHOD OF INOCULATING SUNFLOWERS WITH DOWNY MILDEW, AND SOLUTIONS TO SOME OF THEM

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SUMMARY

Leaf disks may be difficult to wet. Seven surfactants tested in whole seedling immersion (WSI) inoculations all permitted good infection and sporulation; all but Tween 20 and 80 induced stunting. All treatments improved wetting of disks in LDI; sporulation was best with Tween 20. Sometimes in LDI inoculations sporulation is less than expected in incompatible cultivar–race combinations, or more than expected in incompatible combinations. Decreased sporulation is often associated with profuse bacterial growth. Of eleven readily available antibiotics tested rifampicin appeared best at reducing bacterial contamination without adverse effects on downy mildew. Mold growth was often profuse on disks when bacterial competition was reduced by rifampicin treatment. In spite of these difficulties, the LDI method is proving useful in various studies, and efforts are being made to resolve the problems.

Key words: Downy mildew, inoculation method, sunflower diseases, leaf disk immersion

INTRODUCTION

The leaf disk immersion (LDI) method of inoculating sunflowers with *Plasmopara halstedii* (Farl.) Berl. & deToni gave *in vitro* reactions in compatible and incompatible cultivar–race combinations comparable to those obtained by the standard whole seedling immersion (WSI) method (Sackston et al 1987, Sackston and Vimard 1988a, 1988b). As a non–destructive test it would make feasible investigations not possible with the destructive WSI test.

Some difficulties have been encountered with the LDI method. In some experiments, very little or no sporulation was observed on leaf disks in compatible combinations, in which heavy infection resulted from WSI inoculations. As leaf disks may be difficult to wet in the inoculum suspensions, an effort was made to improve wetting and increase the probability of infection by adding surfactants to the suspension.

Bacterial growth was often profuse on and around the disks, and fungal contamination also occurred in some cases. Such conatamination was not considered to be important when the method was first developed, as it rarely prevented mildew sporulation completely, and permitted qualitative assessments of presence or absence of the pathogen. When it became necessary to make quantitative assessments of mildew sporulation, however, bacterial interference with the pathogen posed a problem. Various

antibiotics were tested in an effort to control the bacteria without inhibiting the mildew. This paper presents the results of some of our efforts to overcome such problems.

MATERIALS AND METHODS

Sunflower genotypes

Sunflower lines or hybrids used in various tests carried either no known genes for resistance to downy mildew (Peredovik, Krasnodarets, IS 003), or the resistance genes $P1_1$ (CM5RR; CM90RR), or $P1_2$ (RHA 274, apparently plus an additional gene; 894; IS 7000) (Sackston et al 1990, Gulya et al, in press).

Mildew races

The four races of downy mildew used were 0; 1; 1,2; 1,2,5CL; (North American races 1; 2; 3; and 4; respectively) (Sackston et al 1990).

WSI inoculations

WSI inoculations (Cohen and Sackston 1973) were made in all experiments as controls on the viability of the inoculum and the identity of the races. At least two replicates of 10 pregerminated seedlings of each cultivar were incubated for 3 hours at 15°C in a suspension of the respective races containing about 30,000 zoosporangia per ml. They were then planted in a commercial soil substitute containing peat, vermiculite, and fertilizer, and grown under controlled conditions until the first true leaves were well expanded, before sporulation was induced in a saturated atmosphere.

LDI inoculations

LDI inoculations were made using 10mm disks cut with a sterile cork borer from well expanded first true leaves of plants grown to the two pairs of leaves stage in the commercial soil substitute, under controlled conditions. Disks were immersed in suspensions of 30,000 to 50,000 zoosporangia for 3 hours at 15C. Ten disks per dish were then placed on 1% water agar in 90mm petri dishes under controlled conditions for 12 to 14 days; preliminary observations were made after 7 or 8 days. At least two plates were used for each treatment for each cultivar/race combination in each experiment. Experiments were repeated various numbers of times.

Surfactants

Seven sufractants were tested at three concentrations each for their effect on plant development and infection in WSI inoculations of zoosporangia, and effect on sporulation of the pathogen in LDI inoculations. They were dissolved in water, then added to spore suspensions to obtain the final concentrations: agar, at 0.1, 0.05, and 0.01% by weight/volume; gelatin at 0.25, 0.1, and 0.05% by weight/volume; household dishwashing detergent; Bond (Carboxylated synthetic Lalex); Sorbo (Sorbitol solution); Tween 20 (Polyoxyethylene sorbitan monolaurate); and Tween 80 (Polyoxyethylene sorbitan monooleate), all at 0.05, 0.01, and 0.005% volume/volume.

Antibiotics

Eleven antibiotics were tested in preliminary experiments for their effect on bacteria and on zoosporangium germination in suspensions of *P. halstedii*, on mildew infection in WSI and in LDI inoculations, and on bacterial contamination of disks in LDI inocualtions. They were: chloramphenicol*, claforan, garamycin (gentamycin)*, neomycin, novobiocin*, penicillin G*, pivampicillin, rifampicin*, streptomycin*, tetracycline*, and vancomycin, at concentrations of 10 and 100 ppm except for gentamycin, which was applied at 16 and 80 ppm. Eight of them indicated by (*), were amond the 23 tested on the pathogen *in vitro* by Oros and Viranyi (1988). We selected five antibiotics for further testing at four concentrations.

RESULTS AND DISCUSSION

Surfactants

Mildew infection was normal in all tests by WSI with all surfactants except the detergent at the highest concentration, which inhibited sporulation. Height of seedlings was reduced in the series treated with the highest concentration of agar, the medium and high concentrations of Bond and Sorbo, and by all concentrations of gelatin. Tween 20 and Tween 80 had no apparent effect.

All surfactant treatments improved wetting of leaf disks; the disks did not sink in the spore suspensions, but the sufaces were obviously wet. All treatments with detergent prevented sporulation on the disks. The highest concentration of Bond reduced sporulation; all the other treatments increased the sporulation compared to the inoculum without surfactants. All except the detergent also increased mold growth on the disks, thus limiting their usefulness.

Antibiotics

In the preliminary tests of the antibiotics and untreated controls in zoosporangium suspensions of each of the four mildew races, gentamycin, neomycin, rifampicin, streptomycin, and tetracycline all greatly reduced the number of bacterial colonies in streaks of the suspension on the surface of potato dextrose agar (PDA) in petri dishes. Gentamycin, neomycin, novobiocin, streptomycin, and tetracycline inhibited or prevented mildew infection in WSI and/or sporulation in LDI inoculations, so were eliminated from further tests. Novobiocin gave relatively poor control of bacteria; streptomycin and tetracycline were somewhat phytotoxic. Penicillin did not affect mildew development, but was eliminated as it had no apparent effect on the bacterial contaminants.

In subsequent tests chloramphenicol was used at 10, 100, and 500 parts per million (ppm); pivampicillin (pondocillin) at 10, 100, 250, and 500 ppm; rifampicin at 10, 25, 50, and 100 ppm; and vancomycin at 10, 50, and 100 ppm. Zoosporangial suspensions were incubated at 15 °C for 3 hours for most of the zoospores to emerge before the antibiotics were added to the inoculum. Rifampicin at all concentrations tested gave the best control of bacteria in streaks on PDA and on disks in LDI tests and had no apparent effect on mildew development in WSI inoculations. It did not inhibit mildew sporulation on disks in LDI when added at 10, 25, or 50 ppm, but reduced sporulation at 100 ppm. There was much more sporulation on the surface of the disks than on the edges in rifampicin

treatments, whereas most of the sporulation was confined to the edges in the controls and other antibiotic treatments. Mold growth was much more conspicuous on disks in the rifampicin treatments than in the others. Results of preliminary experiments indicate that some of the mold contamination arose from the leaf disks, but that much of it was introduced with the mildew inoculum. Apparently the removal of bacterial competition in the rifampicin treated disks permits the increased mold growth.

CONCLUSIONS

There are problems and weaknesses to be overcome in the LDI inoculation method. It has given some interesting results in spite of its imperfections, however. Results of LDI inoculations using leaves of adult plants showed clearcut restriction of downy mildew from cultivated sunflower and from an ornamental species to the host of origin (Sackston 1989). In tests with some incompatible host genotype – mildew race combinations in 1987–1988 (Sackston, unpublished) where sporulation occurred on the cotyledons but not on the leaves in WSI, results were the same in LDI, suggesting that resistance was physiological. In others, where sporulation occurred on disks from the leaves as well as from the cotyledons, failure to infect the leaves in WSI might be attributable to the existence of a barrier at the cotyledonary node (Montes and Sackston 1976).

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PROBLEMAS CON EL METODO DE LA INMERSION DEL DISCO FOLIAR (LDI) PARA LA INOCULACION DE GIRASOLES CON MILDIU, Y SOLUCIONES A ALGUNOS DE AQUELLOS

RESUMEN

Los discos foliares pueden ser difíciles de humedecer. Los siete mojantes ensayados en las inoculaciones por inmersión completa de plántulas (WSI) permitieron buena infección y esporulación; todos ellos excepto Tween 20 y 80 indujeron enanismo. Todos los tratamientos mejoraron el humedecimiento de discos en LDI; la esporulación fue mejor con Tween 20. La esporulación en las inoculaciones por LDI es a veces menor que la esperada en combinaciones compatibles cultivar–raza, o mayor que la esperada en combinaciones incompatibles. La disminución de esporulación está frecuentemente asociada con extenso crecimiento bacteriano. De los once antibióticos de fácil disponibilidad que se ensayaron, rifampicina pareció el mejor para reducir la contaminación bacteriana sin efectos adversos sobre mildiu. El crecimiento de hongo fue con frecuencia intenso en discos cuando la competición bacteriana se redujo por el tratamiento de rifampicina. A pesar de estas dificultades, el método LDI se prueba de utilidad en varios estudios, y se están realizando esfuerzos para resolver los problemas.

PROBLÉMES RENCONTRÉS AVEC LA MÉTHODE "IMMERSION DE DISQUES FOLIAIRES" (IDF) APPLIQUÉE À L'INOCULATION DU TOURNESOL PAR LE MILDIOU, RÉPONSES À CERTAINS DE CES PROBLÉMES.

RÉSUMÉ:

les disques foliaires peuvent être difficiles à humidifier. les sept surfactants testés pour l'inoculation de type "Immersion des plantules entières" (IPE) ont tous permis une bonne infection et la sporulation.Tous, exeptés le Tween 20 et le Tween 80 ont provoqué un retard de croissance. Tous les traitements ont amélioré l'humidification des disques pour l'IDF, la meilleure sporulation étant obtenue avec le Tween 20. Parfois au cours de l'inoculation de type IDF, la sporulation s'est révélée moins intense que nous l'espérions pour les combinaisons races-cultivars ou plus intense dans le cas des combinaisons races-cultivars incompatibles. La diminution de la sporulation était souvent associée à une intense activité bactérienne. Sur les onze antibiotiques testés , la rifampicin a le mieux controlé la contamination bactérienne sans effets négatifs sur le Mildiou. Le développement des moisissures était souvent intensifié par la réduction de la compétition bactérienne par les traitements à base de rifampicin. Malgré ces difficultés, la méthode LDI s'est révélée utile pour diverses études et des efforts sont en cours pour résoudre ces problémes.