# Absence of a Common Antigen Relationship Between Corynebacterium insidiosum and Medicago sativa as a Factor in Disease Development

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ABSTRACT

No precipitin bands were formed in gel-diffusion tests comparing antigens and antisera from a resistant and susceptible alfalfa host and virulent and avirulent isolates of *Corynebacterium insidiosum*. Precipitin bands were formed between the homologous combinations included in the same tests and between: the two host cultivars; root and leaf antigens of the two cultivars; and the avirulent and virulent bacterial isolates. Serological differences were not detected when the antigens obtained from gnotobiotically and greenhouse-grown alfalfa were compared in gel-diffusion tests. Antigens obtained from crude host tissue extracts did not react with bacterial antisera, further demonstrating the lack of common antigens between the host and pathogen. No precipitin pattern shifts occurred when antigens from infiltrated and control leaves of a resistant and a susceptible cultivar were compared against host and bacterial antisera. The media and manner in which the pathogen was grown, prior to antigen preparation, had a pronounced effect on serological results obtained.

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The specificity of the host-parasite relationship as determined by virulence or avirulence of the pathogen and resistance or susceptibility of the host is a complex, intriguing, and much investigated problem. Several studies in this area on animal and human diseases have implicated the importance of common antigens; i.e., antigenic components that are common to both host and pathogen (5, 7, 8, 16, 17, 20, 21, 24, 26, 27, 28, 33). However, a review of the literature on the role of common antigens in plant disease reveals that this potentially important area has been little considered. Alterations in the protein metabolism of the infected plant have been investigated considerably, but only a few attempts have been made to demonstrate a common antigen basis for disease. Some studies which have been conducted indicate that the presence of a common antigen(s) may be an important factor that prevents triggering of the host defense mechanism, thus allowing the pathogen to parasitize the host (4, 6, 9, 26).

Doubly et al. (9, 10) completed the initial study on common antigens in plant disease by making serological comparisons between *Melampsora lini* and four rust-differentiating varieties of flax. Results showed that a specific antigen in each of four races of the fungus was commonly shared by only those lines of flax that were susceptible to a particular race. From their study, these authors concluded that a common protein (antigen) basis may be a critical factor governing the resistance or susceptibility of a plant to a disease-producing organism.

The common antigen basis of host resistance or susceptibility, in a nonobligate pathogen model, was tested by Schnathorst & DeVay (26). They compared the antigenic composition of two races of *Xanthomonas malvacearum*, causing angular leaf spot of cotton, with antigens in three varieties of cotton that differentiate these races. Five other species of *Xanthomonas* were also included in the study. Results showed that both bacterial races shared several antigens with the cotton leaves, but resistant varieties shared fewer antigens than susceptible ones. There was a greater intensity of reaction with antigens of pathogenic bacteria than with those of nonpathogens. It was concluded that antigenic compatability may play a role in governing the resistance or susceptibility of certain varieties of a host for a given pathogen species.

Uritani & Stahmann (32) studied the relationship between the magnitude of disease resistance and antigenic compounds produced by sweet potato infected by *Ceratocystis fimbriata*. They demonstrated a correlation between the amount of a protein component found in several Japanese sweet potato varieties and the magnitude of resistance in root tissues to infection by the fungus.

DeVay et al. (6) studied isolates of *Ceratocystis fimbriata* showing sharply defined specificity for sweet potato and stone fruit trees. Serological results indicated a close relationship between the most virulent isolate from sweet potato and almond and a similar relationship between the resistant and susceptible sweet potato varieties. Of greatest significance was the antigenic similarity of the most virulent isolate on sweet potato and the susceptible sweet potato variety. The authors concluded that an immune response may be functional in plants and that susceptibility or resistance may be dependent, in some host-parasite interactions, on the presence or absence of certain antigens.

More recently, Charudattan & DeVay (4) demonstrated a common antigen relationship among *Fusarium* species and wilt-susceptible and

wilt-tolerant varieties of cotton. When the common antigenic substance was isolated and purified, it was found to be a polysaccharide-protein complex. It was postulated that the common antigen may be involved in the establishment and survival of *Fusarium* isolates in host tissue.

Common antigens which appear to be involved in disease susceptibility have also been shown between host plants and pathogens in the case of crown gall of sunflower and tobacco (25, 27). Wimalajeewa & DeVay (33) have also demonstrated the occurrence of a common antigen relationship between *Ustilago maydis* and *Zea mays*. Characterization of the relationship indicated that the common antigens were associated with the protein components of their ribosomes.

It can be concluded that much more research is needed to validate the common antigen basis for plant disease, to determine whether it occurs in other systems, and to elucidate the exact nature and physiological role of common antigens in the disease process.

A review of the literature related to bacterial wilt of alfalfa indicated that the host-parasite relationship involved might provide a suitable model for investigating the common antigen basis for disease. The bacterium has a sharply defined host specificity. There are only a few reports describing *Corynebacterium insidiosum* (McCull.) H. L. Jens on any host except alfalfa. Also, there are clear varietal differences in reaction of the host to the pathogen.

Many of the studies thus far conducted on common antigens in plant disease have utilized host-pathogen combinations where resistance and susceptibility is governed by a single gene or a few genes controlling the same reaction. To determine whether these combinations are widespread, it was desirable for us to study a system where this was not the case. Again, bacterial wilt of alfalfa appeared to provide a good model, since different alfalfa varieties consist of very diverse genotypes, and resistance to *C. insidiosum* appears not to be controlled by a single gene. However, varieties at opposite ends of the spectrum do not overlap greatly in phenotypic expression in resistance or susceptibility. Therefore, the primary objective of these studies was to determine whether a common antigen basis is operative in bacterial wilt of alfalfa (*Medicago sativa* L.) caused by *Corynebacterium insidiosum*. Other objectives were to determine whether different serological results are obtained when antigens are prepared from greenhouse-grown and gnotobiotically grown alfalfa plants, and the feasibility of using the presence or absence of common antigens as a tool in screening for resistance to *C. insidiosum*.

A major criticism of previous studies on common antigens in plant disease is that the host plants, from which inject antigens were prepared, were not maintained under sterile conditions. Therefore, any microbial population(s) present could have a significant influence on the composition of the inject antigens and serological results obtained with their use. The present study was designed to prevent this factor by growing plants under gnotobiotic conditions.

MATERIALS AND METHODS.—*Growth of plants*.—Alfalfa plants, cultivars DuPuits (highly susceptible) and Vernal (highly resistant), were used because their reaction to bacterial wilt has been well documented (11, 12, 30).

Plants were established and maintained under gnotobiotic conditions, using germ-free isolators and techniques similar to those reported by Lukezic et al. (19).

Alfalfa seeds used in these studies came from the foundation seed lots. We sterilized them by soaking in 75% ethanol for 10 min followed by 20 min in 0.2% (w/v) aqueous mercuric chloride solution. They were then rinsed in sterile distilled water. Several preliminary tests on different media had indicated this was the best method for seed sterilization. Subsamples of these seeds were placed on several different media prior to introducing the remainder into the isolator where the final rinses were completed. Seeds in the isolator were germinated on potato-dextrose agar (PDA) plates and allowed to grow for 10 days to check for any microorganisms. Sterile seedlings were removed and planted in washed river-bank sand. Plants were watered from the bottom by use of Hoagland's solution No. 1 (15) which we modified to reduce salt accumulation by reducing $KNO_3$ to one-half the recommended amount and omitting $MnCl_2$. The pH of the solution was adjusted to 6.5 by addition of sterile 0.1 N KOH. Since the plants were maintained for a year, it was still necessary to flush the sand periodically with sterile distilled water to prevent injury due to salt accumulation.

Light intensity, provided by a bank of fluorescent bulbs supplemented with incandescent bulbs, was 10,900 lux at plant height. Plants were routinely clipped back to a height of 5-8 cm as they approached the flowering stage.

We conducted sterility tests periodically by placing samples of plant tissue and sand on PDA, Czapek-Dox agar (Difco), beef-lactose agar (Difco), nutrient agar, nutrient broth, and liquid thioglycollate medium (Difco). Samples on each medium were incubated at 21 and 32 C for several days to check for any contaminating microorganisms (14).

Another set of plants used in these studies were maintained in the greenhouse for the same period. They were clipped at the same time as the chamber plants, and were otherwise treated in as similar a manner as possible.

*Preparation of host antigens*.—Antigens were prepared from DuPuits and Vernal alfalfa, grown under gnotobiotic and greenhouse conditions, by a method similar to that of Uritani & Stahmann (31). Plants were divided into two fractions for extraction: (i) leaves and stems; and (ii) roots and crowns. One-hundred grams each of leaf and root tissue was used from 32 plants of each variety grown under both conditions. All extraction procedures were carried out in the cold (0-4 C).

The final dialyzate obtained by this extraction procedure was concentrated in half by the use of Lyphogel (Gelman Instrument Co., Ann Arbor, Mich.). The concentrated solution was divided into subsamples and frozen until needed.

The presence of ascorbic acid in the extraction solution prevented the use of standard protein determination techniques. Therefore, a method using Ponceau-S fixative dye was adapted for use with solutions containing ascorbic acid. We determined protein concentration by spotting 5 $\mu$liters of the antigen on a cellulose acetate strip. The same amount of a standard, crystalline human albumin serum (Nutritional Biochemicals Corp., Cleveland, Ohio) was then spotted on the same slide at concentrations ranging from 0.5 mg/ml to 10 mg/ml in increments of 0.5 mg/ml. The slide was stained for 10 min in Ponceau-S fixative dye and rinsed three times (1 min/rinse) in 5% acetic acid (Millipore Corp., Bedford, Mass.). We determined protein concentrations by comparing intensity of antigen spots with those of the standard.

To determine if some host protein(s) that might react with bacterial antisera were lost by the centrifugation and/or dialysis steps involved in the handling procedure, antigens were also extracted from greenhouse plants of both cultivars using the same buffer. However, this crude extract was not processed any further but frozen until needed for serology tests.

Since a previous study indicated that avirulent cells of *C. insidiosum* could induce protection against virulent cells in infiltrated leaves (3), an experiment was completed to determine whether a change in antigens was involved. Approximately 400 leaves (5 g of tissue) of Vernal and DuPuits alfalfa were infiltrated with avirulent cells of *C. insidiosum*. An equal amount of control leaves were infiltrated with water. Leaves were harvested 12 hr later, and proteins were extracted by the technique previously given. The final dialyzates, after concentration, were stored frozen until needed for serological tests.

*Preparation of bacterial antigens.*—Antigens were prepared from *C. insidiosum* by a method similar to that of Lucas & Grogan (18). The entire procedure was carried out under sterile conditions and at 0-4 C.

Beef-lactose agar (BLA) plates were seeded by streaking the entire surface of the plate with a glass rod dipped in a suspension of the bacteria. An agar medium was used for increasing the bacterium, since it has been reported to form gums in shake culture and these might influence the antigen preparations (13, 29). After 10 days' growth at 21 C, 15 plates of each isolate were flooded with 10 ml of sterile physiological saline and scraped gently to loosen the cells. The resulting suspension was filtered through a Kimwipe (Kimberly Clark Corp., Neenah, Wisc.) and stirred for 20 min. The cells were sedimented by centrifugation at 10,000 *g* for 15 min, resuspended in sterile physiological saline, and centrifuged again. This process was repeated 3 times.

The material was then sonicated until the milky cell suspension became opalescent (0.5 hr at a setting of 7 on a Branson Model IS-75 sonifier). Following sonification, the suspension was concentrated in half by the use of Lyphogel, and protein determinations were completed. The samples were subdivided and frozen until needed.

Inject and test antigens were thus prepared from avirulent and virulent cultures of *C. insidiosum* (S-5 D) that were initially derived from the same single-celled isolate as previously described (1). Test antigens were also prepared from isolates derived from the virulent pink and avirulent green colony types found on tetrazolium chloride in a previous study (2), from two other avirulent-virulent single-cell combinations of *C. insidiosum* obtained from isolates G-1 F and S-5 B, and from a gram-positive, small rod bacterium (N-1) isolated from wilt infected alfalfa. The latter produced typical wilt symptoms in experimentally inoculated alfalfa, but failed to produce any of the typical blue pigment when cultured on BLA.

Antigens were also prepared in the same manner from an avirulent and virulent isolate of *C. insidiosum* (S-5 D) that was grown in shake culture according to the method of Spencer & Gorin (29). This results in the formation of gums which the authors have related to disease development. These antigen preparations were used in serology tests to determine if any component(s) were formed in shake culture but not on BLA, whether the gums interfered with antigen production and/or preparation, and whether any fractions associated with the gums were antigenic in nature.

To determine whether *C. insidiosum* might be stimulated to form any different antigenic component(s) on a medium containing host tissues, the following medium was used: 100 g of alfalfa in distilled water was ground in a Waring Blendor and filtered through four layers of cheesecloth. This was made up to 1 liter after combining with 5 g glucose, 5 g peptone, and 15 g agar. This was sterilized by autoclaving for 20 min. Four separate media were thus prepared using leaf-stem and root-crown fractions of both Vernal and DuPuits alfalfa. Both an avirulent and virulent isolate of S-5 D were grown on the different media, and antigens were prepared for serology tests as previously described.

*Preparation of antisera.*—Antisera was prepared against an avirulent and virulent isolate (S-5 D) of *C. insidiosum* and the leaf and stem fraction of DuPuits and Vernal alfalfa grown under gnotobiotic conditions.

Rabbits, weighing 4-5 pounds, were starved for 24 hr, and preimmunization serum was collected. Two rabbits each were injected with the four different antigens. The initial injection consisted of 0.25 ml of antigen in the marginal ear vein plus 2.00 ml (emulsified with 2.00 ml of Freund's incomplete adjuvant) in the hip muscle. Injections in the marginal ear vein started at 0.25 ml and were increased by 0.25 ml at 3-day intervals until 1.5 ml was reached. Rabbits were bled from the marginal ear vein 4 days after this last injection. After 7 days' rest, 1.75 ml was injected into the marginal ear vein and 2.0 ml

into the hip muscle (again emulsified with adjuvant). Four days later, blood was collected by the method of cardiac puncture. Rabbits were always starved for 24 hr before collection of blood. Collected blood was kept at room temperature for 1 to 2 hr. Clots were then gently loosened and stored overnight at 4 C. Antisera was then decanted and clarified by low-speed centrifugation. It was subdivided into small samples, placed in serum vials, and stored frozen, without preservative, until needed. It was held at 4 C when in use.

*Serological tests.*—Antibody titer of the different antisera was determined by the extinction dilution method. We employed the Ouchterlony double-diffusion method (22, 23) to determine serological relationships among the various antigens and antisera. Gel-diffusion tests were made in 9-cm plastic petri dishes containing 15-20 ml of 1-1/2% Special Noble agar (Difco) in phosphate saline buffer (pH 7.0-7.5) (4.0 g NaCl, 0.7 g $Na_2HPO_4$, 0.6 ml 1.0 N HCl in 500 ml $H_2O$). Orange G (0.006%) was added to provide a better contrasting background, and 0.05% $NaN_3$ was added as a preservative. Plates were freshly prepared as needed, and allowed to dry 24 hr before use. A circular pattern with six outer wells (0.7 cm in diam, 1 cm apart, and 1 cm from the central well) surrounding a central well (1-cm-diam) were cut in the agar with a Feinberg agar-gel cutter (Colab).

Bacterial and host antigens, along with antisera, were prepared to the desired concentration by dilution with sterile physiological saline. These were placed in the wells in various combinations, incubated at either 21 or 37 C, and protected from drying by sealing in a plastic bag. Wells were refilled once or twice at 3- to 4-day intervals with the antigens and antisera. A few drops of sterile physiological saline was then added to each well. Plates were incubated for as much as 2 weeks and placed at 4 C for several more days.

RESULTS.—Gel-diffusion tests, completed with preimmunization sera for interaction with test antigens, were negative.

Titer of the antisera (reciprocal of the dilution) collected after both injection periods, ranged from 5,120 for the bacterial antisera to 10,240 for the host antisera.

Protein concentration of the antigens prepared from Vernal and DuPuits leaves, grown under both sets of conditions, was 3.0 to 4.0 mg/ml; the concentration of the root antigens was 1.0 to 2.0 mg/ml. Antigens prepared from the avirulent and virulent isolates of *C. insidiosum* were approximately 1.0 mg of protein/ml.

The gel-diffusion tests to determine the interaction between host-pathogen combinations of antisera and antigens were negative, as precipitin bands were not formed in these heterologous combinations (Fig. 1, 2). However, several bands were formed in the homologous combinations included in the same tests. Similar results were obtained when antigens prepared from two other isolates of *C. insidiosum* (G-1 F and S-5 B) were tested in the same manner. These results were also confirmed by tests in which the reactants were placed in different combinations to show the exact relationship of the bands to each other (Fig. 3). Antigens from the virulent pink and avirulent green colony types of *C. insidiosum*, detected on the tetrazolium medium, did not react with host antigens in any test.

There was little difference in the pattern of bands formed between either avirulent or virulent antisera and their homologous and heterologous antigens.

Antigens obtained from host roots formed fewer bands than those prepared from leaves. Root antigens did not form any bands with the avirulent and virulent antisera, but did react with both the homologous and heterologous host antisera (Fig. 2).

Tests to determine whether there were any differences in antigens prepared from greenhouse and gnotobiotically grown alfalfa did not detect any differences in the leaf or root antigens prepared from either category of plants.

Results of tests on the crude extracts obtained from host tissue indicate some additional bands not found in tests with the dialyzed extracts (Fig. 4). However, in no case did the antigens in the crude extract react with the bacterial antisera.

No protein band shifts occurred when antigens from infiltrated and control plants were compared against host and bacterial antisera by the gel-diffusion method (Fig. 5). Several bands were formed in the homologous combinations. Again, however, no precipitin bands were formed between these host antigens, and the bacterial antisera thus showing the lack of common antigens between the two.

The medium and manner in which the pathogen was grown, prior to antigen preparation, had a pronounced effect on serological results obtained. When the bacterium was grown in shake culture or on media containing host tissue, the number of precipitin bands was greatly reduced in comparison to those grown on BLA. This was true for both the avirulent and virulent isolates.

Antigens prepared from the white bacterium (N-1) causing typical wilt symptoms in alfalfa formed a minor band against Vernal and DuPuits leaf and virulent *C. insidiosum* antisera in gel-diffusion tests. It did not react with avirulent *C. insidiosum* antiserum.

DISCUSSION.—Results obtained in this study demonstrate that common antigens are not involved in bacterial wilt of alfalfa caused by *C. insidiosum*. Gel-diffusion tests with pathogen and host antisera and antigens failed to demonstrate any common precipitin bands between the heterologous combinations. The results obtained are in contrast to other studies on the role of common antigens in plant disease (4, 6, 9, 10, 25, 26, 27, 33).

We should state that extraction methods and the gel-diffusion system used in this investigation are different from those used by Charudattan & DeVay (4), DeVay et al. (6), Schnathorst & DeVay (26), and Wimalajeewa & DeVay (33), who have detected common antigens between parasites and their hosts. However, we feel that the techniques used in this study and the results obtained are valid, because of

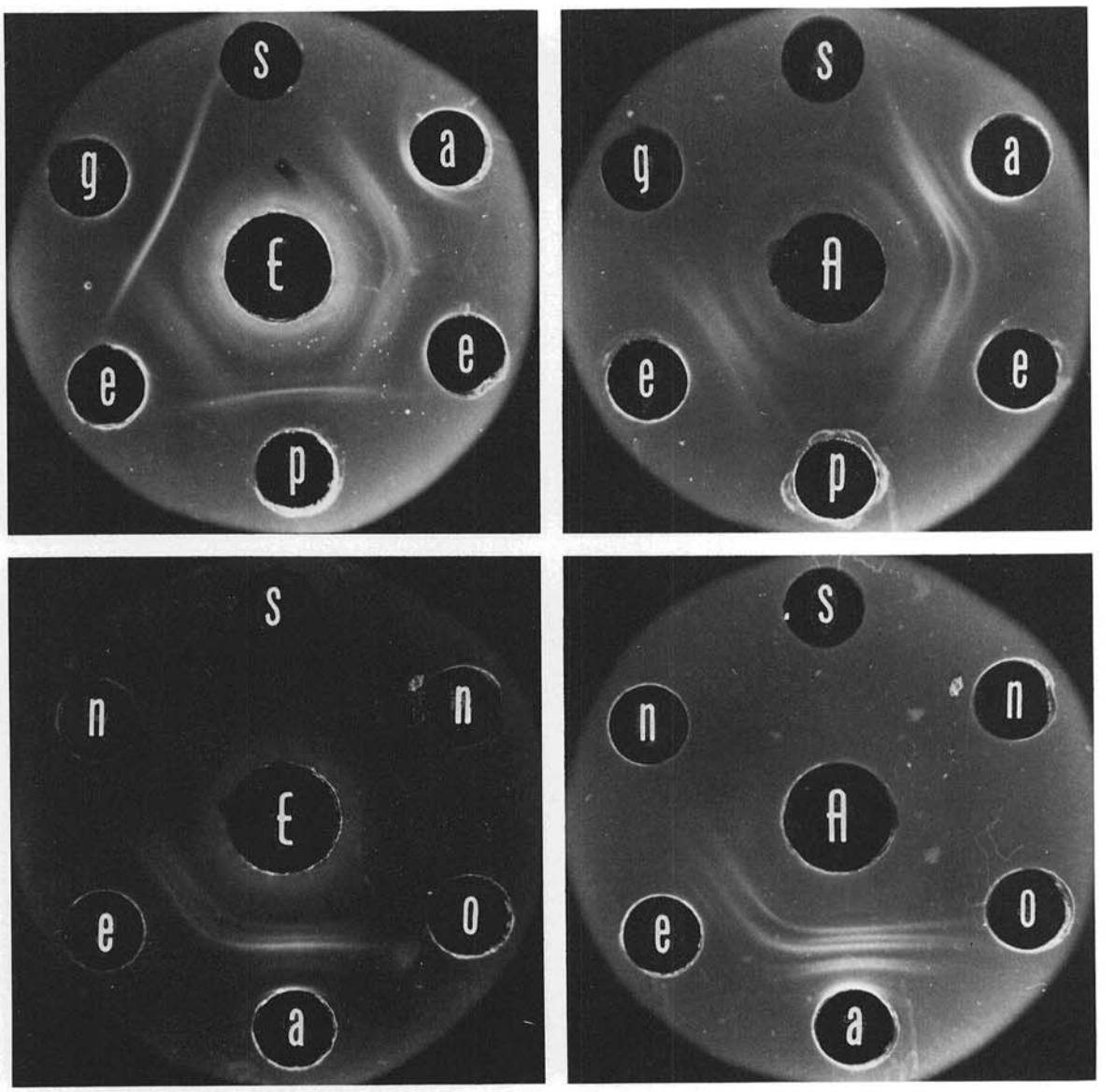


**Fig. 1.** Precipitin band pattern in gel-diffusion tests showing the relationship of bacterial antisera to bacterial antigens (*Corynebacterium insidiosum*) and the lack of response to a host antigen (*Medicago sativa*). Antigens (in outer wells): s = saline; a = avirulent; e = virulent; p = from virulent pink colony type; g = from avirulent green colony type; n = DuPuits leaf; o = Vernal leaf. Antisera (in center wells): E = virulent; A = avirulent.

the success reported by others using a similar system (32) and the band formation that we obtained in the gel-diffusion tests (Fig. 1, 2).

That no antigens in the crude extract reacted with the bacterial antisera further confirms the lack of any common proteins between the host and pathogen, and shows that no host antigens, which may have reacted with the pathogen, were lost due to centrifugation and dialysis of host protein extracts.

The lack of any difference between antigens prepared from the greenhouse and gnotobiotically grown host suggests that a major criticism directed toward previous studies on common antigens in plant disease may be groundless. Plants grown in the greenhouse, in the presence of a normal microbial population, and those grown in the gnotobiotic chamber were the same with respect to their antigenic components.

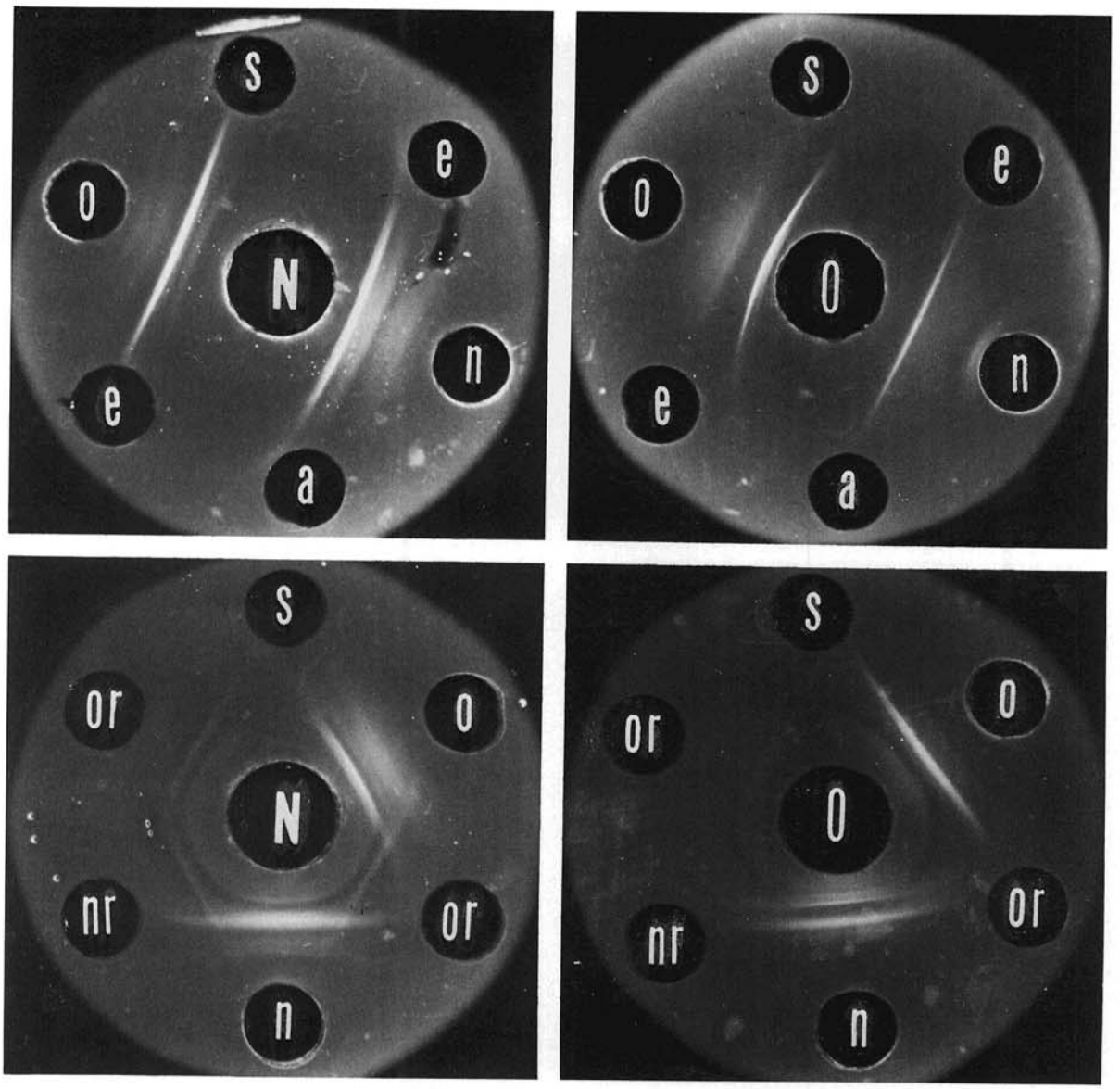


**Fig. 2.** Precipitin band pattern in gel-diffusion tests showing the relationship of host antisera to host antigens (*Medicago sativa*) and the lack of response to *Corynebacterium insidiosum* antigens. Antigens (in outer wells): s = saline; a = avirulent; e = virulent; or = Vernal roots; nr = DuPuits roots; N = DuPuits leaf; O = Vernal leaf.

The lack of any precipitin band shift due to infiltration of leaves with avirulent *C. insidiosum* cells indicates that an antigenic relationship between the host and pathogen is not responsible for the induced resistance observed in a previous study (3).

It is significant that the antigens of the virulent pink and avirulent green cell types of *C. insidiosum* detected on the tetrazolium chloride medium never interacted with the host antisera in gel-diffusion tests. The white bacterial isolate (N-1) is serologically closely related to isolate S-5 D because it formed several bands with the S-5 D antiserum. It is interesting that antigens from this white isolate did form a precipitin band with the host antisera. However, at this time the band must be considered an artifact, since it occurred between both the resistant and susceptible host antisera.

The fact the bacterium failed to show a similar antigenic structure when grown on different media points out the importance of the media on the antigenic properties of the bacterium. This appears not to be important in this investigation because, when bacteria grown on the different media were tested, they remained either virulent or avirulent and

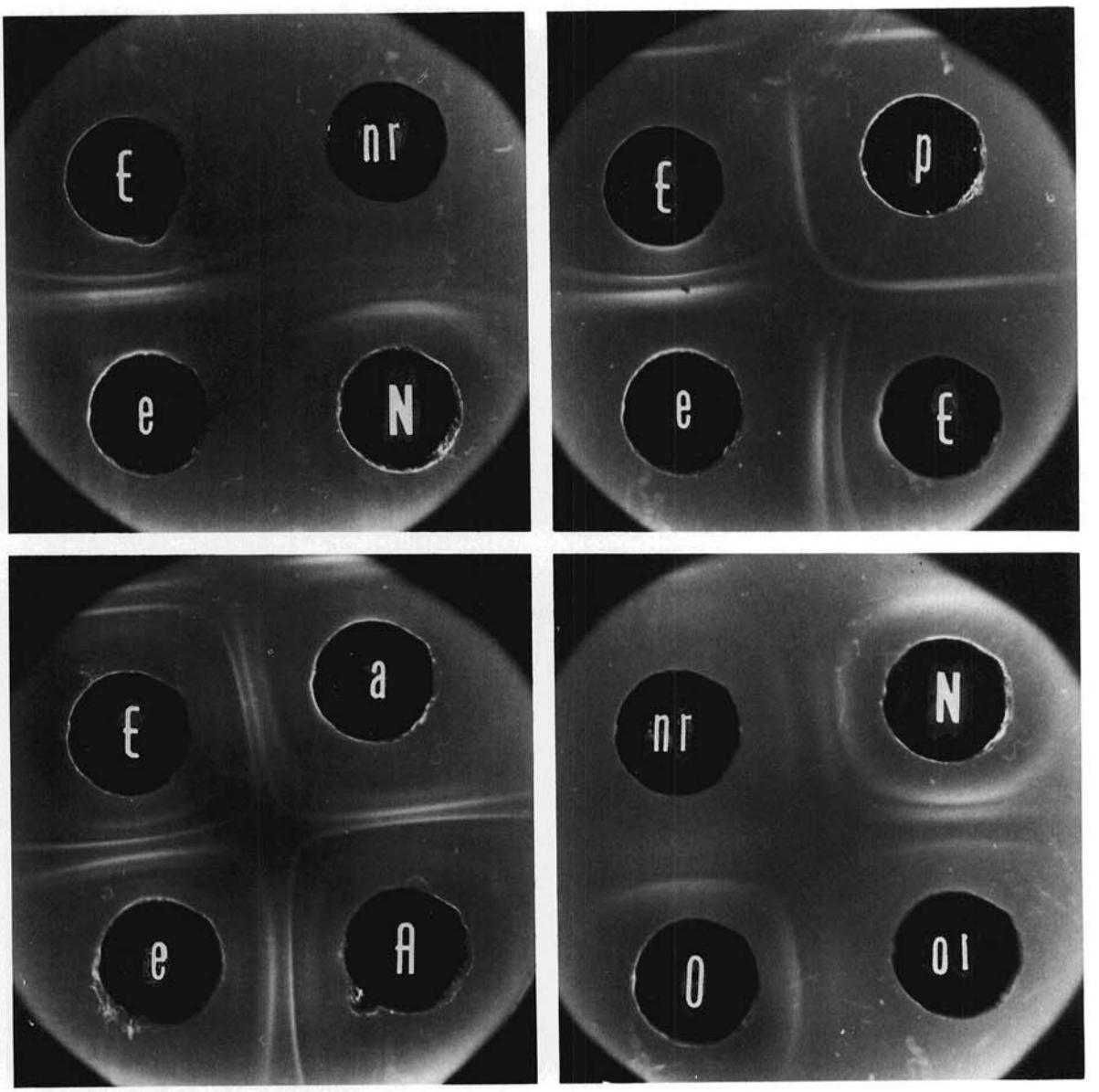


**Fig. 3.** Precipitin bands in gel-diffusion tests employing a different well pattern to illustrate the relationship of host (*Medicago sativa*) and bacterial (*Corynebacterium insidiosum*) antisera to antigens. Antigens (in outer wells): a = avirulent; e = virulent; p = virulent pink colony type; nr = DuPuits roots; or = Vernal roots. Antisera (in center wells): A = avirulent; E = virulent; N = DuPuits leaf; O = Vernal leaf.

the host reaction was not altered. In shake culture, the antigens were either not formed or were interfered with in serological tests by the presence of gums. The gums were not antigenic in nature with host tissue, and were formed by both the virulent and avirulent culture.

Based on the information available from this investigation, common antigens do not have a role in resistance of alfalfa to *C. insidiosum*. The difference in results obtained in this and previous studies may be due to the difference in the models employed. Many of the previous studies have utilized a host-pathogen combination where resistance is governed by a single gene or a few genes controlling the same reaction, whereas this study did not. The genetics of inheritance in alfalfa is very complex as it behaves as an autotetraploid. Also, different alfalfa varieties are very heterogeneous. Further studies on host-pathogen combinations will be required to determine if common antigens are involved in other plant diseases of this nature.

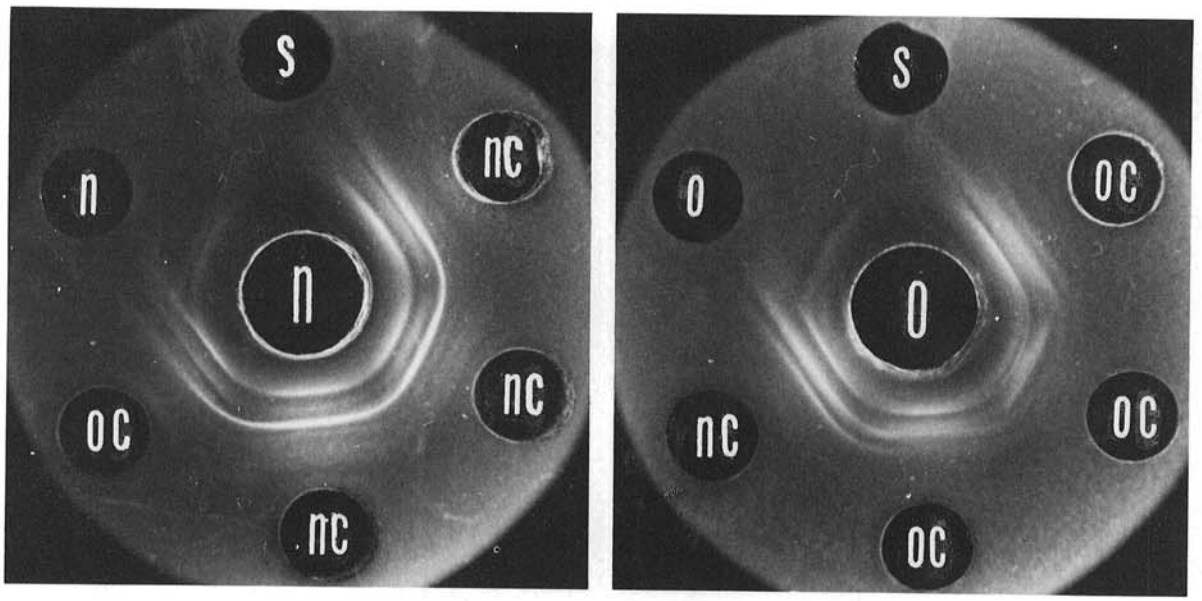


Fig. 4. Precipitin band pattern in gel-diffusion tests resulting from the interaction of host antisera (*Medicago sativa*) with antigens in a crude host extract. Antigens (in outer wells): s = saline; n = DuPuits leaf; o = Vernal leaf; nc = DuPuits leaf (crude extract); oc = Vernal leaf (crude extract). Antisera (in center wells): N = DuPuits leaf; O = Vernal leaf.

## LITERATURE CITED

1. CARROLL, R. B., & F. L. LUKEZIC. 1971. Preservation of Corynebacterium insidiosum in a sterile soil mix without loss of virulence. Phytopathology 61:688-690.
2. CARROLL, R. B., & F. L. LUKEZIC. 1971. Methods of preservation of Corynebacterium insidiosum isolates in relation to virulence and colony appearance on a tetrazolium chloride medium. Phytopathology 61:1423-1425.
3. CARROLL, R. B., & F. L. LUKEZIC. 1972. Induced resistance in alfalfa to Corynebacterium insidiosum by prior treatment with avirulent cells. Phytopathology 62:555-564.
4. CHARUDATTAN, R., & J. E. DE VAY. 1972. Common antigens among varieties of Gossypium hirsutum and isolates of Fusarium and Verticillium species. Phytopathology 62:230-234.
5. DAMIAN, R. T. 1967. Common antigens between adult Schistosoma mansoni and the laboratory mouse. J. Parasitol. 53:60-64.
6. DE VAY, J. E., W. C. SCHNATHORST, & M. S. FODA. 1967. VI. Protein metabolism in the host. Common antigens and host-parasite interactions, p. 313-328. *In* C. J. Mirocha & I. Uritani [ed.]. The dynamic role of molecular constituents in plant-parasite interactions. Bruce Publishing Co., St. Paul, Minn.
7. DINEEN, J. K. 1963. Immunological aspects of parasitism. Nature 197:268-269.
8. DINEEN, J. K. 1963. Antigenic relationship between host and parasite. Nature 197:471-472.
9. DOUBLY, J. A., H. H. FLOR, & C. O. CLAGETT. 1960. Relation of antigens of Melampsora lini and Linum usitatissimum to resistance and susceptibility. Science 131:229.
10. DOUBLY, J. A., H. H. FLOR, & C. O. CLAGETT. 1960. Breakthrough on plant disease. Agr. Res. 8:3-4.
11. ELLING, L. J., & F. I. FROSHEISER. 1960. Reaction of twenty-two alfalfa varieties to bacterial wilt. Agron. J. 52:241-242.
12. FROSHEISER, F. I., & L. J. ELLING. 1965. Bacterial wilt reaction and survival of alfalfa varieties in artificial inoculation trials. Crop Sci. 5:368-369.
13. GORIN, P. A. J., & J. F. T. SPENCER. 1961. Extracellular acidic polysaccharides from C. insidiosum and other Corynebacterium spp. Can. J. Chem. 39:2274-2281.
14. HAMLEN, R. A. 1971. Identification of carbohydrates in root exudates of alfalfa, Medicago sativa L. cv DuPuits, grown under gnotobiotic conditions, and their influence on egg hatch of Meloidogyne incognita (Kofoid & White) Chitwood. Ph.D. Thesis, Penn. State Univ. 79 p.
15. HOAGLAND, D. R., & D. I. ARNON. 1950. Water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Circ. 347, 32 p.
16. JENKIN, C. R., & D. ROWLEY. 1959. Bactericidal activity of macrophages in vitro against Escherichia coli. Nature 181:1738-1739.
17. KAPLAN, M. H. 1965. Induction of autoimmunity to heart in rheumatic fever by streptococcal antigen(s) cross-reactive with heart. Federation Proc. 24:109-112.
18. LUCAS, L. T., & R. G. GROGAN. 1969. Serological variation and identification of Pseudomonas lachrymans and other phytopathogenic Pseudomonas nomenspecies. Phytopathology 59:1908-1912.
19. LUKEZIC, F. L., J. R. BLOOM, & ROSLYN G. LEVINE. 1969. Influence of top removal on the carbohydrate levels of alfalfa crowns and roots grown in a gnotobiotic environment. Can. J. Plant Sci. 49:189-195.
20. LYAMPERT, I. M., O. I. VVEDENSKAYA, & T. A. DANILOVA. 1966. Study on streptococcal group A

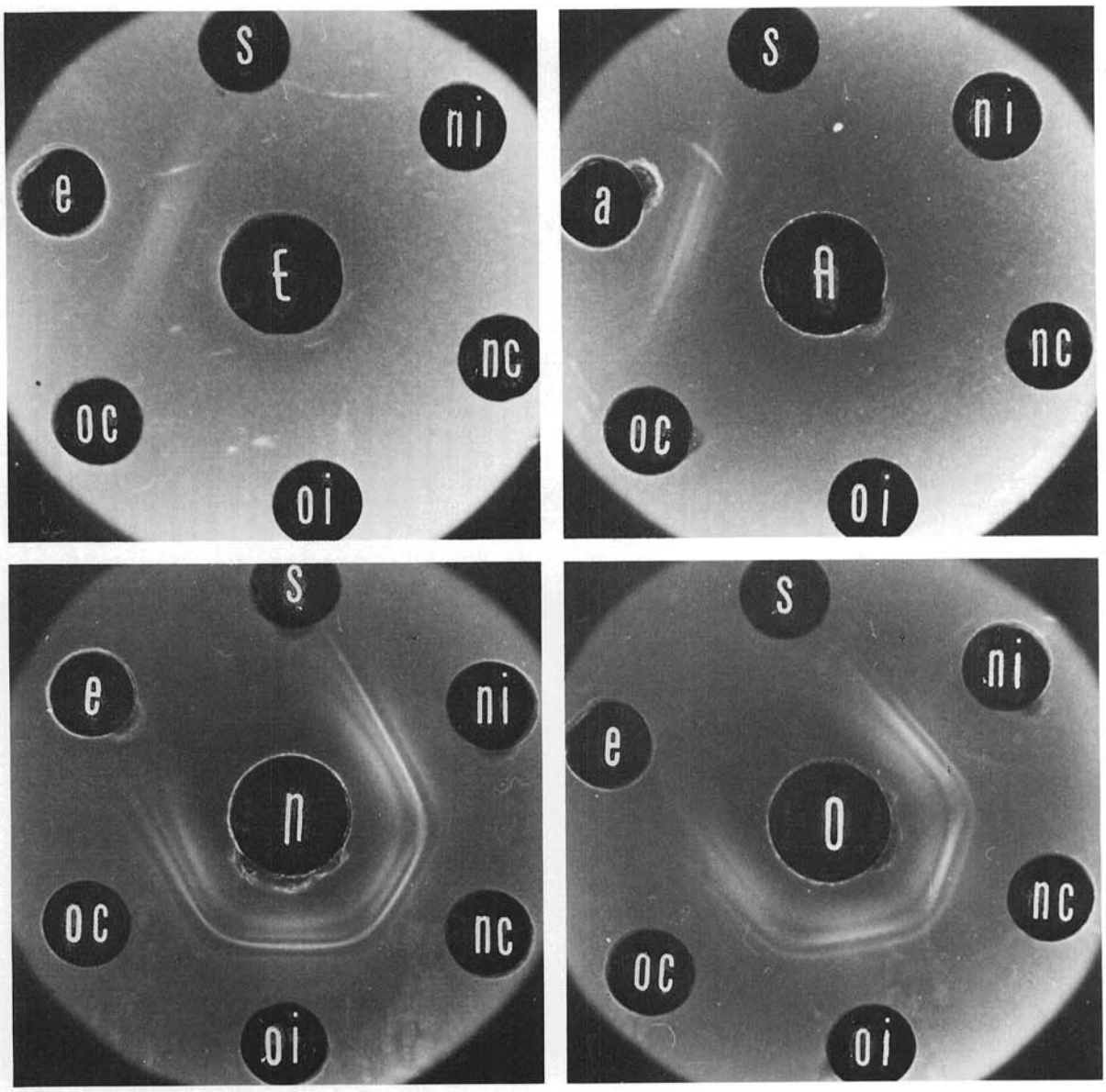


**Fig. 5.** Precipitin band pattern in gel-diffusion tests resulting from the interaction of host antisera (*Medicago sativa*) and antigens obtained from inoculated and control plants 12 hr after infiltration with *Corynebacterium insidiosum*. Antigens (in outer wells): s = saline; a = avirulent; e = virulent; ni = DuPuits leaf inoculated; nc = DuPuits leaf control; oi = Vernal leaf inoculated; oc = Vernal leaf control. Antisera (in center wells): A = avirulent; E = virulent; N = DuPuits leaf; O = Vernal leaf.

antigens common with heart tissue elements. Immunology 11:313-320.

21. MARKOWITZ, A. S., S. H. ARMSTRONG, JR., & D. S. KUSHNER. 1960. Immunological relationships between the rat glomerulus and nephritogenic streptococci. Nature 187:1095-1097.

22. OUCHTERLONY, O. 1953. Antigen-antibody reactions in gels. IV. Types of reactions in coordinated systems of diffusion. Acta Pathol. Microbiol. Scand. 32:231-240.

23. OUCHTERLONY, O. 1958. Diffusion-in-gel methods for immunological analysis. *In* S. K. Basel [ed.]. Progress in allergy 5:1-78.

24. ROWLEY, D., & C. R. JENKIN. 1962. Antigenic cross-reaction between host and parasite as a possible cause of pathogenicity. Nature 193:151-154.

25. SCHILPEROORT, R. A., W. H. MEIJS, G. M. W. PIPPEL, & M. VELDSTRA. 1969. Agrobacterium tumefaciens cross-reacting antigens in sterile crown gall tumors. FEBS (Fed. Evr. Biochem. Soc.) Lett. 3:173-176.

26. SCHNATHORST, W. C., & J. E. DE VAY. 1963. Common antigens in Xanthomonas malvacearum and Gossypium hirsutum and their possible relationship to

host specificity and disease resistance. Phytopathology 53:1142 (Abstr.).

27. SCHNATHORST, W. C., J. E. DE VAY, & T. KOSUGE. 1964. Virulence in Agrobacterium tumefaciens in relation to glycine attenuation, host, temperature, bacteriophage and antigenicity to pathogen and host. p. 30-32. *In* J. E. DeVay & E. E. Wilson [ed.] Conf. Abnormal Growth Plants Proc., Univ. of Calif., Berkeley.

28. SIMONSEN, M., & R. J. C. HARRIS. 1956. Actively acquired tolerance to heterologous antigens. Acta Pathol. Microbiol. Scand. 39:21-34.

29. SPENCER, J. F. T., & P. A. J. GORIN. 1961. The occurrence in the host plant of physiologically active gums produced by Corynebacterium insidiosum and Corynebacterium sepedonicum. Can. J. Microbiol. 7:185-188.

30. UNITED STATES DEPARTMENT OF AGRICULTURE. Crops Research Division, Agriculture Research Service. 1962. Alfalfa varieties and areas of adaptation. Leaflet No. 507.

31. URITANI, I., & M. A. STAHMANN. 1961. Changes in nitrogen metabolism in sweet potato with black rot. Plant Physiol. 36:770-782.

32. URITANI, I., & M. A. STAHMANN. 1961. The relationship between antigenic compounds produced by sweet potato in response to black rot infection and the magnitude of disease resistance. Agr. Biol. Chem. 25:479-486.

33. WIMALAJEEWA, D. L. S., & J. E. DE VAY. 1971. The occurrence of a common antigen relationship between Ustilago maydis and Zea mays. Physiol. Plant Pathol. 1:523-535.