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**Pathogenicity and Sclerotial Development of
Sclerotinia sclerotiorum: Involvement of Oxalic
Acid and Chitin Synthesis**

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Pathogenicity and Sclerotial Development of *Sclerotinia sclerotiorum*:

Involvement of Oxalic Acid and Chitin Synthesis

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II. ABSTRACT

Title: Pathogenicity and Sclerotia Development of *Sclerotinia sclerotiorum*: Involvement of Oxalic Acid and Chitin Synthesis

Sclerotinia sclerotiorum (Lib.) de Bary is among the world's most successful and omnivorous fungal plant pathogens. Included in the nearly 400 species of plants reported as hosts to this fungus are canola, alfalfa, soybean, sunflower, dry bean, and potato. The general inability to develop resistant germplasm with these economically important crops to this pathogen has focused attention on the need for a more detailed examination of the pathogenic determinants involved in disease development. A mechanistic understanding of the successful strategy(ies) used by *S. sclerotiorum* in colonizing host plants and their linkage to fungal development may provide targets and/or novel approaches with which to design resistant crop plants. This proposal involved experiments which were successful in generating genetically-engineered plants harboring resistance to *S. sclerotiorum*, the establishment and improvement of molecular tools for the study of this pathogen and the analysis of the linkage between pathogenicity, sclerotial morphogenesis and two biosynthetic pathways: oxalic acid production and chitin synthesis. The highly collaborative project has improved our understanding of *S. sclerotiorum* pathogenicity, established reliable molecular techniques to facilitate experimental manipulation and generated transgenic plants which are resistant to this economically important fungus.

III. ORIGINAL OBJECTIVES

1. To determine the potential of oxalic acid degradation by plants susceptible to *S. sclerotiorum* as a mechanism of engineered resistance against this and other fungal pathogens.
- II. To analyze the involvement of oxalic acid production and chitin synthesis in sclerotial formation of *S. sclerotiorum*.

The broad objectives are:

- 1) To develop molecular tools to advance studies of this fungus.
- 2) To test the idea of interfering with compatibility determinants (in this case,

oxalic acid) as an approach to generating durable transgenic disease resistant plants.

IV. OVERVIEW OF ACHIEVEMENTS

In the original proposal our objectives were to (i) generate transgenic potato harboring an “oxalase” gene and evaluate such plants for resistance to *S. sclerotiorum*, (ii) develop molecular tools to better study this fungus and analyze the involvement of oxalic acid and chitin synthesis during sclerotial formation by this fungus. All of these objectives has not only been addressed, but importantly have also been advanced.

A. System Development

A primary focus of our research efforts has been the development of molecular tools and reagents to advance present and future studies of *S. sclerotiorum*. Towards this we have accomplished the following during the granting period. First and foremost has been the development and improvement of DNA mediated transformation for this fungus. Our continued efforts to improve transformation have resulted in a 50 to 100-fold improvement in transformation efficiency.

Two factors have contributed significantly to the improvement of transformation efficiency: (i) improved methods of culturing and protoplasting *S. sclerotiorum* mycelia, and (ii) the use of transformation vectors containing autonomous replicating sequences (ARS) derived from *Fusarium oxysporum*. This ARS sequence contains telomere repeat sequences and vector derived inverted repeats. Two concerns associated with the introduction of telomere repeats into the genome are that transformants may be unstable when taken off selection, and that vectors containing telomere sequence have the potential to “break” chromosomes if they become integrated into the genome. We have found that transformants are >90% stable when propagated off and then back onto selective media. Molecular analysis of transformants by Southern hybridization indicates that this stability is due to integration of the vector into the genome. Integration of *Fusarium* ARS vectors has also been reported. In *S. sclerotiorum* nonintegrative copies of the vector are also sometimes observed in conjunction with integrated copies but are apparently lost upon successive vegetative propagation. Southern hybridization analyses using telomere repeat sequences as a probe to look

for RFLPs between individual transformants and the recipient strain have failed to detect polymorphisms among endogenous telomere sequences. This indicates that transformants retain all of their chromosome ends intact, i.e. chromosome arms distal to the integration site are not lost. The increased efficiency of *S. sclerotiorum* transformation to 100-200 transformants per µg of DNA is a major accomplishment for the further molecular genetic development of *S. sclerotiorum* and is an essential tool for the proposed work.

Two wild-type genomic libraries of *S. sclerotiorum* have been constructed, a EMBL-3 genomic library and a cosmid library. Prior to constructing a wild-type cosmid library, a high efficiency cosmid vector containing the *Fusarium* ARS sequences had to be constructed. To this end, a unique PvuII site in pMOcosX was filled-in and ligated to the blunt ended 1.3 kb *Fusarium* ARS sequence. This new cosmid vector transformed *S. sclerotinia* to hygromycin resistance at a rate of ca. 150 transformants per µg of vector DNA. This represents a 100-fold increase in efficiency from the original pMOcosX. Importantly, cosmid clones with large (avg. 35 kb) inserts retain high transformation frequencies. Southern hybridization analysis has demonstrated that transforming pMOcosTEL DNA is stably integrated into the genome. This cosmid library is currently being used to directly complement Oxa::Scl- mutants of *S. sclerotiorum* and will be utilized in the proposed research for identifying genomic copies of cDNA clones. To date we have screened 2000 transformants; none have shown complementation for either OA or sclerotial development. These studies are continuing.

Expression vectors containing *Fusarium* ARS sequences, the hygromycin phosphotransferase (*hph*) cassette, and the *A. nidulans* *gpd* promoter and *trpC* terminator separated by a multiple cloning site have been constructed. This construct allows for forward subcloning of genes into an expression vector with a constitutive promoter and retains high efficiency transformation of *S. sclerotiorum*. This vector has been tested and shown to transform *S. sclerotiorum* with high efficiency and is capable of driving expression of the -glucoronidase (*gus*) gene which can be histochemically detected as X-gluc stained hyphae (Rollins and Dickman unpublished data). Moreover, we recently have been able to successfully express the green

fluorescent protein (GFP) in this fungus which will greatly enhance our future studies. Thus, a reporter gene system for evaluation of promoter/regulatory sequences in *S. sclerotiorum* is in place.

The isolation of high quality nucleic acids is essential for molecular genetic analysis of an organism. Accordingly, numerous protocols have been developed for the routine isolation and purification of DNA and RNA from various species of fungi. Nevertheless, fungi vary in their physical and biochemical growth habits making it necessary to optimize extraction procedures for the organism of interest. *S. sclerotiorum* produces large quantities of extracellular carbohydrates when cultured, making the purification of nucleic acids somewhat troublesome. Although others have reported nucleic acid extraction procedures for *S. sclerotiorum*, these have limitations in their requirements for lyophilized tissue, the use of low quantities of fungal tissue to avoid excessive carbohydrate contamination or the necessity for preparation of protoplasts. We have modified these procedures to isolate DNA from *S. sclerotiorum* with good results. Additionally, we have developed new procedures which utilize proteinase K incubation and organic solvent extraction to decrease carbohydrate contamination and increase DNA yields. This procedure has been scaled down such that all grinding and extraction steps can be carried out in 1.5 ml eppendorf tubes thus allowing numerous samples to be processed simultaneously in a short amount of time. Additionally, high quality RNA can be isolated from *S. sclerotiorum* mycelia by the standard procedures. These procedures allow the use of fresh or lyophilized tissue, eliminate excessive carbohydrate contamination, and allow larger quantities of tissue to be used than previously reported procedures, thus, increasing DNA and RNA yields. These optimized protocols will be used routinely in the proposed studies and will facilitate day-to day progress.

Methods to synchronize sclerotia development over a uniform area of fungal tissue have been developed. Our method is similar to that used to obtain synchronized sclerotia development in *Sclerotium rolfsii*, with slight modifications. We first attempted to synchronize sclerotial formation in cultures of this pathogen on lactose-containing Joham's medium, in which we observed that sclerotia initials are formed rather synchronously. Nonetheless, we were not content

with this form of induction and therefore proceeded to seek alternative means for synchronously producing sclerotia in culture. We have now concluded that transferring a culture of the fungus from a submerged liquid to drier growth conditions induces the massive synchronous production of sclerotial initials. Technically, this is done by filtering a liquid culture on Whatmann #1 paper over a Büchner funnel. The filter paper (on which the mycelial mat is resting) is transferred to a petri dish containing 5mm glass beads set in liquid medium. The filter paper serves as a wick, supplying nutrients to the culture, which is now mostly exposed to air. Within 24-48 hrs massive sclerotial initials are formed. This method will also be used to generate a sclerotia specific cDNA library, currently under way in the Dickman lab, which will be shared with the Yarden lab and made available to the scientific community.

Thus, our efforts to establish a transformation system, genomic libraries, and uniform conditions for studying sclerotia development not only provide the necessary framework for carrying out the proposed molecular genetic studies of *S. sclerotiorum*, but also the necessary tools for accomplishing our current and future goals.

B. Generation of Transgenic Plants Resistant to *S. sclerotiorum*

Molecular Cloning and Plasmid Construction. The original plasmids, containing putative oxalyl-CoA decarboxylase genes were obtained by PCR from the bacterium *Oxalobacter formigenes*. The PCR generated 1.9 kb fragments were subcloned into two binary plant expression vectors downstream from the cauliflower mosaic virus 35S promoter (CaMV 35S) or the Chlorella virus adenine methyl transferase gene promoter (amt). The resultant constructions achieved transcriptional fusion between the promoters and putative oxalase genes. The binary plasmids contained a neomycin phosphotransferase plant selectable marker gene driven by the T-DNA gene nopaline synthase promoter and a transcriptional termination signal sequence from the T-DNA transcript 7 gene. The binary plasmids were used to produce transgenic potato plants by *Agrobacterium*-mediated transformation. Putative transformants, growing on kanamycin, were selected and transferred to regeneration media. Five stably transformed plants have been grown in our greenhouse. We have developed an in vitro testing procedure for potato leaf sensitivity to *S.*

sclerotiorum. The test is comprised of placing agar mycelial plugs of the pathogen on detached leaves or leaf segments placed on 0.03% Benzimidazole agar. The inoculated leaves are incubated under light and within 3-5 days pathogen proliferation and tissue necrosis can be observed. When leaf segments are inoculated with the sclerotia/oxalic acid defective mutants, little, if any at all, necrosis occurs. Using both a detached leaf assay and whole plants, varying levels of resistance to *S. sclerotiorum* were obtained. The partial resistance was characterized by both reduced disease severity as well as a delay in the onset of symptoms. Even though the resistance conferred to the transgenic plants did not reach the levels observed following inoculation with the oxalic acid-deficient *S. sclerotiorum* mutants, we believe that the reduced symptoms or delay in symptom development may have significant agricultural value. We are currently in the process of applying for a patent disclosure for this work. Furthermore, these results demonstrate that the idea of interference with compatibility determinants, via generation of transgenic plants, is likely to be a useful approach with attractive features (e.g. durability) specifically for *S. sclerotiorum* and generally for other pathogens where such determinants are known (For details, see reference 3). This and other transgenic strategies have the potential to successfully control *Sclerotinia* diseases and demonstrates how knowledge of basic disease mechanisms can lead to novel ideas for controlling disease. Further work on defining the genetic and biochemical bases of fungal development and pathogenesis may lead to the development of more diverse, effective, long-term and ecologically safe disease control methods.

C. Sclerotia Development and Oxalic Acid

The original oxalic acid deficient mutants were also sclerotia-less. These observations suggest a link connection between OA production and sclerotia morphogenesis. As a starting point to identify the molecular factors which trigger and regulate sclerotia morphogenesis and/or OA production, pharmacological investigations on conserved signal transduction pathways were conducted. Compounds known to increase endogenous cAMP levels in other organisms by inhibiting phosphodiesterase activity (caffeine and 3-isobutyl-1-methyl xanthine) or by activating adenylate cyclase (NaF), reduced or eliminated sclerotia development in *S. sclerotiorum*. Growth

in the presence of 5mM caffeine correlated with increased levels of endogenous cAMP in mycelia. Additionally, including exogenous cAMP or the cAMP analog 8-Br-cAMP in the growth medium decreased or eliminated the production of sclerotia in a concentration dependent manner. Inhibition of sclerotia development was found to be cAMP specific, as exogenous cAMP, AMP, and ATP did not influence sclerotial development. Transfer of developing culture to cAMP containing medium at successive time points demonstrated that cAMP inhibits development prior to or during sclerotia initiation.

Results of this study identified cAMP as an endogenous inhibitor of sclerotia development in *S. sclerotiorum* (and other *Sclerotinia* spp.). Furthermore, from this study, cAMP appears to up regulate oxalic acid synthesis. Our current work has thus demonstrated these high concentrations of cAMP inhibit sclerotia development and simultaneously increase OA production.

This has led us to postulate two models linking sclerotial development and OA biosynthesis. In the first model, cAMP regulates early sclerotial development, and OA metabolism is essential for later sclerotial development. In this model, cAMP regulates early sclerotial development prior to convergence with OA metabolism; however, since OA metabolism is necessary for sclerotial development, a block anywhere in the OA pathway also blocks sclerotial development. Our experimental evidence demonstrates that blocking sclerotial development with exogenous cAMP results in strict vegetative growth and increased OA accumulation. We speculate that the increase in OA accumulation is a result of increased vegetative growth and not directly regulated by a cAMP-dependent pathway. In this model *Oxa-::Scl*-mutants could arise by a single mutation in the OA biosynthetic pathway prior to convergence with the sclerotial development pathway.

The second model proposes that OA metabolism and sclerotial development are part of a bifurcating pathway which branches prior to OA biosynthesis or cAMP-dependent regulation of sclerotial development. As in the first model, high concentrations of cAMP would inhibit sclerotial development and indirectly increase OA accumulation. In this model, a single block in the OA metabolic pathway prior to divergence with the cAMP-mediated sclerotial development pathway

Strategy for sequencing the *SsclhA1* gene fragment. The arrow heads indicate the direction in which the fragments were sequenced. Internal regions of pOE1 were sequenced with oligonucleotide primers (*PstI1* - *PstI6*). Sequenced subclones are designated by the appropriate restriction site used for subcloning.

10	30	50	70	90
TACCATATCCCAGTTTTTCAGGCCGGGAGGAAAGnAGGGATAGGGGTGGGAATTATTGATTGTAACnGACACATCAACATACAACGTTGTAGTCAACC				
110	130	150	170	190
TTACAATCTAGTACGGAAGAAGGCTTGGATCGAAAGATATTCCTCAAAnTGAGCCCTTCGCAAGGAAATTCATGTGGCTCnTACGCATCCCTTCACCCCA				
210	230	250	270	290
CAGATCaCAATAGGACGGTAATCTCATACAACGGTACCATGTCTGATATAATCGATTAGGTATGTCTCTCTATCTAATTTACCAAATCTCTTCTCCACCTTC				
310	330	350	370	390
CTAGCACACITTTGTCCGCGTTTCGCATTGTGATGGCAGTGAAGTGAATGTAAAGTTTGAATCAAATCACACATTGTCTTGAAGACCATTACCGTCGACCA				
410	430	450	470	490
GTGTATCCATTCTGCGGCGCATAGCAACCAACACGAAAGGCGCAACCAAGTAAACTATACGCGCAAAGAGGATTTCTTGATGGGTGCGAGAATTACTTG				
510	530	550	570	590
ATACACTCAGTTTCCCTCTGCTAGGACAGAAGCAGCTTTTCTCTCTATCAACAAATCATCTGCAATATCTACACTTTTGTATTATCTACAGCAAACGTTT				
610	630	650	670	690
CTAATATTTCGACCCACACAGATCCGGCGGGCCAAGGCCAAGACGATTATTACGACATGAACAATAGACACAACCTCGCGCGTCTCAAGGATACCAACTG				
710	730	750	770	790
GAAGATGCGCCATATGGAAGACAACCTATTGGTTACCCGGTCTGGTATTCCCAATCTGGAGATACCTATGGGCCCTGGAGGACACAGAGTTGGAACAC				
810	830	850	870	890
CAAGTGATCAATTACAGCCTCAGCCATCTGTGAGTTTTTAGGTCGATGTGCCTGCGAAGAGATGAAGCTTTATCTGACATGAATTCTACAGTATTCCGTC				
910	930	950	970	990
GAGCATATAGATCAAAGTCAATACCATCAGCGCATGCCCCCTAAATCTCAGTCAAAGTTACGACTCGGGATACTCTTTAGATCCTAACGCACATCAAGATG				
M P L N L S Q S Y D S G Y S L D P N A H Q D A				
1010	1030	1050	1070	1090
CTTACTACCAACCTCCATACCAACCATCTCTCATGAAGAACATCCTTTACAAAATTATCCTCAAGGGCAAGACCCCTACGGTTACAATGACGATGATGA				
Y Y Q P P Y Q P S P H E E H P L Q N Y P Q G Q D P Y G Y N D D D D				
1110	1130	1150	1170	1190
CCATCAACCAATTCTACAGTCGCATGAACCATATGGGCCAGATCCACACACAGCCAGTGGTGCAGAGTATAAAGGTTACGACGGAGCTGGACACTCTCCA				
H Q P I L Q S H E P Y G P D P H T A S G A E Y K G Y D G A G H S P				
1210	1230	1250	1270	1290
TCTTCGACACCTATACCTGCTTTAAGAAGATACAAGACAGTAAAGGAAGTCCAGTTGTTCAATCGAAATCTCGTACTTGATTGTCCAATTCCCCCTAAGC				
S S T P I P A L R R Y K T V K E V Q L F N G N L V L D C P I P P K L				
1310	1330	1350	1370	1390
TTTTAAGTCAAGTCAATCATGCACCGCTCCCGAGAGAGATGAATTTACACATATGAGATACTCAGCGGCAACATGCGATCCAGCGAGTTTTTTGAGGA				
L S Q V N H A P P P E R D E F T H M R Y S A A T C D P S E F F E E				
1410	1430	1450	1470	1490
CGGTTTTACATTGCGACAAAACTCTTTGCAAAGCCTCGACATACAGAACTCTTCATTGTTGTGACTATGTATAATGAAGATGATGTATTGTTGCCCCGA				
R F T L R Q K L F A K P R H T E L F I V V T M Y N E D D V L F A R				
1510	1530	1550	1570	1590
ACGATGCATGGAGTATTCAAGAACGTTGAATATATGTGCACTCGCAAGGATAGTAAACATGGGGAAAGGATGCTTGGAGAAGATCGTAGTGTGCGTTG				
T M H G V F K N V E Y M C T R K D S K T W G K D A W K K I V V C V V				
1610	1630	1650	1670	1690
TCAGTGATGGACGTGCTAAGATCAATCCGAGAACCAGAGCTGTGTTGGCTGGTTTGGGAGTGTACCAAGATGGAATTGCGAAGCAACAAGTTAATGGCAA				
S D G R A K I N P R T R A V L A G L G V Y Q D G I A K Q Q V N G K				
1710	1730	1750	1770	1790
GGACGTGACAGCACATATCTACGAATATACGACGCAAGTTGGCATTGCTCTGAAGAAGGATATCGTTACACTCACACCGAAACAGCAGCCTATTCAACTA				
D V T A H I Y E Y T T Q V G I A L K K D I V T L T P K Q Q P I Q L				
1810	1830			
CTTTTCTGTTTGAAGGAAAAAATCAGAAAAAGATCCGTT				
L F C L K E K N Q K K I R				

Fig. The partial nucleotide sequence of the gene *SschsA1* of *Sclerotinia sclerotiorum* including the 5' flanking region. The proposed sequences of CAAT, TATA Boxes and initiation of translation are underlined. Conserved

isolated clone consists of 910 bp. TATA, CAAT sequences and a potential intron were identified. An interesting feature of the gene is the presence of a putative intron (identified on the basis of highly conserved splice signals) directly upstream of the predicted translation initiation codon. As it is now clear that fungal chitin synthases are regulated at the transcriptional as well as the post-translational levels, the presence of this intron may be an indication for the presence of post-transcriptional regulation as well. The SschsA1 codon preference was found to be with a high bias for AT at the third position (62% of the codons). A relatively high number of codons containing Adenine at the third position (30%) may indicate that SschsA1 is a gene which is not highly expressed. SschsA1 was compared to other chitin synthases from classes I-III and found to be most similar to class I chitin synthases (90% identity to the class III Ncchs-3 gene of *Neurospora crassa*). As mentioned above, another *chs* -conserved region gene fragment belonging to a class I chitin synthase was isolated from *S. sclerotiorum* and its predicted amino acid sequence is 80% identical to the conserved region of SSCHSA1. Due to the high degree of similarity between the 2 gene fragments, we have concluded that they are both class I chitin synthases. The second class I *S. sclerotiorum chs* gene has been designated SschsA2. A multiple alignment analysis, comparing the partial predicted amino acid sequences of the two class I *S. sclerotiorum* genes with other fungal class I chitin synthases is presented in Figure 4. The presence of two class I chitin synthases (a rare phenomenon in filamentous fungi) raises the possibility that this class of chitin synthases may play an important role in *S. sclerotiorum*. As part of the joint effort to study chitin synthases in this fungus, we are now conducting experiments with the goal of determining the consequences of SschsA1 gene disruption. Attempts for isolating additional chitin synthases from *S. sclerotiorum* are under way.

2. Analysis of the effects of chitin synthase inhibitors on *S. sclerotiorum* growth and sclerotia formation. Utilizing the sclerotial-production initiation synchronization procedure we have adapted for this fungus, we have been able to determine that cell walls of mycelium which was induced to form sclerotia contained higher amounts of chitin compared to cell walls which were constantly maintained in liquid medium. In cell wall samples analyzed 3-4 days after sclerotia

formation induction, chitin content was 20-60% higher than in samples prepared from liquid medium-grown cultures. These results show that an increase in cell wall chitin content accompanies sclerotia formation in *S. sclerotiorum*.

The effect of chitin synthesis inhibition on growth of *S. sclerotiorum* was tested. Growth rate of mycelium was inversely correlated with the concentration of Edifenphos (a non-competitive inhibitor) and B (a competitive inhibitor). From the log-probit transformation, values of 50% mycelial growth inhibition (EC₅₀) were calculated. The EC₅₀ values of Polyoxin B and Edifenphos were determined to be 1.9 μ M and 70 μ M, respectively. The number and dimensions of sclerotia formed in the presence of polyoxin B or Edifenphos in the medium, was inversely correlated with the inhibitor concentration (with the exception of a low concentration of Polyoxin B, as described below-figures 5-8). The germination of sclerotia produced in the presence of the inhibitors was apparently unaffected.

Polyoxin B at concentration of 8 μ M induced sclerotia formation. At this concentration, the number of sclerotia produced was three fold higher than that produced in the presence of Polyoxin B at a concentration which is an order of magnitude lower (Fig.6). It is possible that this finding may have implications concerning the study of morphological changes induced by low drug concentrations as well as some problems concerning chemical control (as a result of non-even fungicide application).

***E. Sclerotinia sclerotiorum* as a causal agent for diseases in herb plants.**

Even though our major efforts, throughout this grant period, have been invested in laboratory and greenhouse analyses of the biology of *S. sclerotiorum* and Sclerotinia-potato interactions, we have also been involved in analyses of the occurrence of this pathogen in the field. Specifically, we are interested in monitoring the presence of *S. sclerotiorum* on crops in which this pathogen has not been a major problem to date. The rationale behind this approach is that early identification of such potential problems (in new crops or crops which have been subjected to changes in the growth practice) will assist in future detection processes and development of control measures. The increase in herb production has been accompanied by the inevitable import of new pests and

A

The average diameter of *Sclerotinia sclerotiorum* sclerotia formed with the presence of chitin synthase inhibitors Polyoxin B and Edifenphos in the medium. Bars indicate standard error.

B

Sclerotinia sclerotiorum hyphal growth rate in the presence of the chitin synthase competitive inhibitor Polyoxin B. The difference in colony size measurements did not exceed 15%.

Growth rate of *Sclerotinia sclerotiorum* and the average number of sclerotia formed in the presence of Polyoxin B. Bars indicate the standard error.

The average number of *Sclerotinia sclerotiorum* sclerotia formed (per petri dish) in the presence of the chitin synthase inhibitors Polyoxin B and Edifenphos in the medium, after 6 days of growth. Bars indicate the standard error.

Sclerotinia sclerotiorum hyphal growth rate in the presence of the chitin synthase non-competative inhibitor Edifenphos. The difference in colony size measurments did not exceed 15%.

diseases and by an increase in the occurrence of existing pathogens on these crops, some of which already have devastating effects on herb production. Chemical pest management in herb crops is problematic. Avoiding or minimizing the use of pesticides is especially required with these crops due to the restriction on pesticide residues on the marketable produce. Therefore, it is essential to prevent the occurrence of the primary inoculum. This can be done by regulation of production and transfer of propagation material, seed disinfestation, suppressive growing media, and soil and substrate disinfestation. During the course of this study, *S. sclerotiorum* has been identified as an infecting agent of several herb crops, spanning diverse botanical families. Furthermore, in sweet basil and tarragon, we identified the first case of *S. sclerotiorum* infection in Israel. The occurrence of *S. sclerotiorum* in sweet basil is of special concern, as this is the major herb crop grown in Israel. Though *S. minor* has been reported to infect sweet basil in the United States, there is no reference to the occurrence of *S. sclerotiorum* on sweet basil nor on tarragon in the United States.

V. COLLABORATION

Overall, both partners have experienced this project as a highly collaborative close contact effort. Dickman has visited the Yarden lab on three separate occasions, Yarden has visited the Dickman lab twice. In addition, both P.I.'s have met at the Fungal Genetics Conference (Asilomar, California, US) in the spring of 1995 and 1997. Thus in addition to E-mail, FAX and "regular" mail, the P.I.'s have physically interacted on a number of occasions, which has proven most beneficial. For example, when Dickman initially visited the Yarden lab this past summer (1998) a recently submitted BARD grant was formulated and written. In addition, sharing of clones, antibodies, primers, vectors and strains have occurred throughout the duration of this proposal. Besides the physical exchanges, the exchange of ideas and approaches greatly enhanced the spirit of experimentation and the advancement of the systems.

VI. LIST OF PUBLICATIONS

1. Rollins, J.A., and Dickman, M.B. 1998. Increase in endogenous and exogenous cyclis

AMP levels inhibits sclerotial development in *Sclerotinia sclerotiorum*. Appl. Env. Micro. 64:2539-2544.

2. Gamliel, A., and Yarden, O. 1998. Diversification of diseases affecting herb crops in Israel accompanies the increase in herb crop production. *Phytoparasitica* 26:53-58.
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