Effects of the *Phomopsis convolvulus* Conidial Matrix on Conidia Germination and the Leaf Anthracnose Disease of Field Bindweed (*Convolvulus arvensis*)

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**ABSTRACT**


The conidia-free conidial matrix of *Phomopsis convolvulus* contains 6% dry matter which consists of approximately 89% carbohydrate and lesser amounts of protein and free amino acids. Conidia densities of 10⁶ per milliliter or greater in the presence or absence of matrix are unable to germinate without dilution. Increasing concentrations from 0 to 5% (v/v) of conidial matrix in water reduces conidia germinations from 70 to 11%. Conidia survival in terms of percent germination was greatest (80-100% for up to 9 wk) when conidia were stored at room temperature in the presence of liquid matrix, whereas drying was generally lethal to conidia. The matrix had no effect on the initiation and subsequent development of the leaf anthracnose disease of field bindweed as measured by dry weight accumulation of treated plants. The undiluted conidial matrix of *P. convolvulus* functions to prolong conidia viability and prevent germination of diluted fresh conidia.

Field bindweed (*Convolvulus arvensis* L.) is a troublesome perennial weed in many agricultural areas of the world (21). Control of this weed is difficult with combinations of conventional methods, such as cultivation and cropping practices, with chemical herbicides providing some control (4,22). These methods, however, have met with only limited success, illustrating the need for development of other methods of control. There is growing interest in the use of plant pathogens to control noxious weeds (20). Recently, Ormen-Nuñez et al. (14,15) demonstrated the pathogenicity of *Phomopsis convolvulus* Ormen to field bindweed. They suggested that the fungus might be suitable for development as a mycoherbicide for the biological control of field bindweed.

In the development of mycoherbicides, it is critical to have a comprehensive understanding of all aspects of the disease and the life cycle of the pathogen. *P. convolvulus* is a Deuteromycetous fungus that reproduces by the production of conidia borne in pycnidia. Sporulation of this fungus is accompanied by the production of relatively large amounts of a viscous conidial matrix. Such conidial matrices, through a variety of mechanisms, generally enhance the survival of conidia (8,13), prevent germination of conidia until they have been adequately diluted or suitable infection sites have been reached (3,6), or serve as virulence factors for pathogenicity (11,12). Such information is not currently available for the conidial matrix of *P. convolvulus*.

We report here the results of studies designed to evaluate the role of the conidial matrix produced by *P. convolvulus* in certain aspects of the etiology of the leaf anthracnose disease of field bindweed.

**MATERIALS AND METHODS**

Preparation of conidia and matrix. Isolate 8412 of *P. convolvulus* was grown on potato-dextrose agar (PDA) for 45-60 days and alpha conidia were harvested as previously described (14). Forty to 50 individual droplets of a conidial suspension (25-50 µl containing approximately 10⁶ conidia per milliliter) were placed on PDA 1.2 medium in petri plates (14). After 2-3 wk of incubation at room temperature (21 °C), 1-cm colonies were produced with numerous pycnidia from which conidia exuded in an estimated 25-50 µl of viscous matrix. Two to 3 ml of matrix plus conidia were collected using a Pasteur pipette, transferred to a centrifuge tube, and diluted 10-fold with distilled water. In most cases, conidia were separated from the matrix by centrifugation at 1,000 g for 20 min. The matrix supernatant was removed and sterilized by 0.2-µm ultrafiltration and the pellet of conidia washed twice by resuspension with distilled water and recentrifugation to remove any residual matrix. Conidia were adjusted to the desired density with a hemacytometer. All manipulations of conidia and matrix were performed under sterile conditions.

**Biochemical analysis of matrix.** Aliquots of the conidial matrix from two separate conidia preparations were each analyzed for protein, carbohydrate, and...
amino acids, and total dry matter (three replicate aliquots for each analysis type). Protein was determined according to Lowry et al (10) with bovine serum albumin as the standard. Carbohydrate was measured using the phenol-sulfuric acid method as described by Hodge and Hofferiter (5) with glucose as the standard, and free amino acid was measured using the modified ninhydrin method of Rosen (18) with glycine as the standard. Total dry matter was determined gravimetrically by weighing aliquots dispensed in triplicate on Whatman No. 1 filter paper tape and dried at 50°C.

Effects of the conidial matrix on conidia germination and survival. To evaluate the effects of the matrix on conidia germination, freshly harvested conidia in native matrix, washed conidia resuspended in various concentrations of matrix diluted with water, or various densities of washed conidia in water alone were placed in cavity microscope slides (100 μl of conidial suspension per cavity) (19). Unless otherwise specified, the standard density of conidia for germination measurements was 2 × 10⁴ per milliliter. After 24 hr at room temperature (21°C), germination was evaluated by counting three microscope fields of approximately 50 conidia each on three slides per treatment. Germination was considered to occur when the germ tube was as long as the width of the conidium.

In preliminary experiments to isolate a germination inhibition, 10-fold diluted conidia-free matrix from original conidia plus matrix volumes of 3–5 ml was extracted three times with equal volumes of ethylacetate. The pooled ethylacetate extracts were dried with a vacuum rotary evaporator and the resultant residue was redisolved with sonication in a volume of water (ambient pH 5.5) equal to that of the original matrix extracted. The effect of this extract on germination of conidia of P. convolvulus was then determined. A similar extract of distilled water was performed as a control. The remaining aqueous fraction was not analyzed.

The effect of the matrix on the survival of conidia (as judged by germinability) was assessed by preparing three conidial suspensions: 1) freshly harvested conidia in native matrix, 2) washed conidia resuspended in matrix diluted 10-fold with water, and 3) washed conidia resuspended in distilled water. The densities of all suspensions were adjusted to the original concentration in native matrix (approximately 10⁷ to 10⁸ conidia per milliliter). Seventy-two 0.1-ml aliquots of each suspension treatment were dispensed into separate vials. The contents of 36 vials of each suspension type were dried under flowing nitrogen at room temperature. Vials containing conidia either dried or in liquid suspension were then stored at room temperature (approximately 21°C, or about 20°C for up to 16 wk). At various time intervals, aliquot vials were assessed for conidia germinability after conidia were resuspended in water and washed by centrifugation and germination measured as described earlier. Alternatively, dried conidia preparations stored at room temperature were first rehydrated in 100% RH by placing water-saturated toweling into sealed vials for 24 hr, after which conidia were suspended in water and germinability was assessed.

Matrix effects on disease. Field bindweed seeds (Valley Seed Co. Fresno, CA) were sown on moist filter paper in petri dishes for 24 hr at 5°C and then germinated at room temperature in the dark for 3 days. Four germinated seeds were sown in Pro-Mix BX potting medium (Las Tourbières Premier, Riviere du Loup, Quebec) in 10-cm plastic pots and thinned to three seedlings per pot after emergence. Plants were grown in growth cabinets with 24/18°C day/night temperature, 14-hr photoperiod, 400 μE·m⁻²·s⁻¹ (14), watered as necessary, and fertilized every 2 wk with 100 ml of Peters fertilizer solution (12.5 g of 20-20-20 per liter) per pot. At the three- to five-leaf stage (10–14 days after planting) all plants in each replicate set (four pots containing three plants each) were sprayed with a total of 4 ml of the desired concentration of matrix per milliliter (0, 10⁻⁶, 10⁻⁷, and 10⁻⁸ conidia per milliliter of matrix diluted 10⁻⁶, 10⁻⁷, 10⁻⁸, and 10⁻¹₀-fold) using two tandem DeVilbiss atomizers (23). Treated plants were then placed in a dark dew chamber for 9, 16, or 24 hr. In two additional experiments to test the effects of delayed dew period, treated plants were returned to the growth chamber for 24 and 40 hr before the dew period. All plants were returned to the growth chamber after the dew period. Fourteen days after the dew period, plants were harvested and disease severity was determined based on plant dry matter accumulation.

Data analysis. Conidia germination data were transformed before analysis with a factorial analysis of variance and regression analysis as appropriate. For regression analyses, treatment sums of squares were broken into single degree of freedom sums of squares, and polynomial regression equations were generated using treatment means and only those terms up to and including the cubic level that were significant at the 5% level of probability. For purposes of the regression analysis, the dilution factor for treatments without matrix was arbitrarily set at 0.0001 so a log scale could be used. All analyses were performed using the McGill University statistical analysis program (PC SAS).

RESULTS

Biochemical analysis of matrix. P. convolvulus matrix contained approximately 6 mg of dry matter per milliliter of native conidia-free matrix (Table 1). Carbohydrate accounted for most (89%) of the dry matter. The matrix also contained approximately 9% protein and less than 1% free amino acids.

Effects of conidial matrix on germination and survival of conidia. No conidia germinated when the density was 10⁶ or 10⁷ conidia per milliliter (Table 2). Germination increased for both washed conidia and conidia in native matrix as the density was decreased from 10⁷ to 10⁶ conidia per milliliter. The factorial analysis of variance indicated a significant (P < 0.05) effect of washing, with germination significantly increased when conidia were washed compared with conidia diluted in native matrix. In another experiment, 49% of washed conidia in water had germinated after 24 hr at room temperature, whereas only 24% had germinated when conidia were incubated in a 1:20 dilution of matrix (Table 3). After 42 hr, germination by washed conidia in water had risen to 95% but conidia in 1:20 matrix had risen to only 35%. When the density of Phomopsis conidia was held constant at 2 × 10⁸ per milliliter, 70% of washed conidia germinated (Fig. 1). Germination decreased as the matrix concentration increased.

Preliminary attempts to isolate a germination inhibitor from the conidial matrice were not successful. Ethylacetate extracts of the matrix redissolved in water inhibited spore germination by up to 50% of control extracts but were never as potent as the native matrix (data not shown).

P. convolvulus conidia survived best when stored without drying and in the presence of native matrix or 1:10 diluted matrix at room temperature (approximately 21°C) (Fig. 2). A factorial analysis of variance of the data for each storage temperature indicated significant (P < 0.05) effects of matrix, drying, storage time, and interaction on survival of conidia, and each treatment was analyzed separately with regression over storage time. Conidia germination before the beginning of the storage periods was 58 ± 14%. When conidia stored at 21°C were sampled after 1 wk, germination had increased from the 58% observed for fresh conidia to 100% for conidia stored without drying and in the presence of native matrix and to 78% for conidia stored without drying and in the presence of 1:10 diluted matrix.
stored without drying and in the presence of 1:10 diluted matrix (Fig. 2). Viability remained high in these two treatments for 7 wk before decreasing. For conidia that were washed and stored in water only, germination remained low and no conidia survived after 7 wk (Fig. 2). When conidia suspensions were dried before storage at 21 C, germination was less than 10% with no germination observed after 3 wk (data not shown).

Except for the water treatment, similar results were obtained for conidia stored at 2 C (Fig. 3). When first sampled 2 wk after being placed in storage, germination was high for all three treatments (native matrix, 1:10 diluted matrix, and washed conidia in water only) stored without drying. Viability decreased with increased storage time, but the presence of native matrix or 1:10 matrix maintained higher viability than water alone. For all treatments dried before storage at 2 C, germination was generally 10% or less and no conidia germinated after 4 wk (data not shown).

Conidia stored dry or in frozen suspension at -20 C generally showed less than 20% germination throughout the storage period (120 days). Conidia in all three liquid suspensions (native matrix, 1:10 diluted matrix, and water) stored frozen had the lowest survival rate with a maximum of 12% germination in native matrix at 60 days with all viability lost by 90 days. Dried conidia from these suspensions, however, maintained 10-20% germination over this storage period (data not shown).

In a separate, more limited experiment, the effects of rehydration of conidia dried in the presence or absence of matrix and stored at room temperature were determined (data not shown). Conidia dried in water and either rehydrated immediately (no storage period) in 100% RH for 24 hr before resuspension in water or resuspended in water without rehydration all showed 0% germination. For conidia dried in the presence of matrix, only those immediately rehydrated in 100% RH showed any germination (3.8 ± 1.3%). However, this was decreased to 0% after storage for 1 wk. The germination of fresh untreated con-

<table>
<thead>
<tr>
<th>Conidia/ml</th>
<th>Spores in native matrix*</th>
<th>Washed spores in water*</th>
</tr>
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<tbody>
<tr>
<td>1 x 10^4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 x 10^5</td>
<td>22 ± 6</td>
<td>68 ± 13</td>
</tr>
<tr>
<td>1 x 10^6</td>
<td>40 ± 2</td>
<td>ND</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>51 ± 12</td>
<td>86 ± 6</td>
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* Values are means of two samples ± standard error of the mean.

Table 2. Effect of *Phomopsis convolvulus* conidia density on germination in the presence of native matrix or water.

![Graph](image1.png)

Fig. 1. Effect of different concentrations of *Phomopsis convolvulus* conidial matrix on germination of conidia at 2 x 10^6 spores per milliliter. Data were arcsine transformed before regression analysis but were back transformed before plotting. Data points represent treatment means. Regression equation: y = -4.35 - 17.77m where y = arcsine transformed germination percentage and m = log matrix dilution factor. R^2 = 0.93.

![Graph](image2.png)

Fig. 2. Germination of *Phomopsis convolvulus* conidia after storage for various times at 21 C without drying. Spores were stored at 10^6 per milliliter, washed, and germinated in water at 2 x 10^6 per milliliter. Data were arcsine transformed before regression analysis but were back transformed before plotting. Symbols represent treatment means. Storage media and regression equations were: native matrix, y = 101.80 - 8.36t, R^2 = 0.84; matrix diluted 1:10 with water, y = 71.14 - 2.89t, R^2 = 0.76; and water, y = 22.14 - 2.35t, R^2 = 0.36. For each equation, y = arcsine transformed germination percentage and t = storage time in weeks.

Table 3. Effect of the conidial matrix on conidial germination after 24 and 42 hr

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>Percent germination</th>
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<tbody>
<tr>
<td></td>
<td>Conidia in 1:20 diluted matrix</td>
</tr>
<tr>
<td>24</td>
<td>24 ± 8</td>
</tr>
<tr>
<td>42</td>
<td>35 ± 3</td>
</tr>
</tbody>
</table>

* Values are the means of three samples ± standard error of the mean. Conidia density was 2 x 10^6 per milliliter and incubation was at 21 C.
conidia in the absence of matrix for this experiment was 89.4 ± 4.1%.

Effects of the conidial matrix on disease development. The conidial matrix had essentially no effect on disease of field bindweed plants as measured by dry weight accumulation of treated plants. When plants were given a 16-hr dew period, there was no significant effect of matrix on uninoculated control plants or on those inoculated with 10³ conidia per milliliter (Fig. 4). Plants inoculated with 10⁶ or 10⁷ conidia per milliliter had slightly increased dry weights when matrix was added compared with those inoculated without matrix (Fig. 4). However, this effect was not apparent when inoculated plants were given 9- or 24-hr dew periods (data not shown). As expected, increased concentrations of conidia resulted in greater disease (less plant dry weight accumulation). Similarly, the conidial matrix had no significant effect on plant dry weight gain when inoculated plants were given delayed dew periods, although the delayed dew period reduced the effectiveness of conidia in causing disease (data not shown).

DISCUSSION

P. convolvulus produces approximately 10⁵ to 10⁶ conidia per milliliter in a viscous matrix which exudes from pycnidia. The conidia-free matrix contains approximately 6% dry matter. This dry matter is composed primarily of carbohydrate with lesser amounts of protein and free amino acids. Although their proportions vary among different species, these substances are common components of conidial matrices of other fungi (2,9,13,16,17). Presumably, conidia may make use of these substances during germination or initial host penetration.

Concentrated conidia in native matrix or water do not germinate without dilution. This is commonly attributable to the presence of natural endogenous inhibitors of germination leached from the conidia or fungal thallus into the matrix (1). The existence of such an inhibitor in the matrix of P. convolvulus seems likely because germination in low concentrations of washed conidia is inhibited in the presence of concentrated matrix or ethylacetate extracts of the matrix. This inhibition is only partly overcome by prolonged germination periods. These observations are consistent with the hypotheses for other fungi (1,6,7) in that the conidial matrix of P. convolvulus also serves to prevent spore germination until a more ecologically favorable distribution has been achieved.

Conidia of P. convolvulus generally lose germinability during storage in water at 20 C, after freezing in the presence or absence of matrix, and storage at −20 C, and especially after drying. Other researchers (13) have suggested that the drying process itself is not particularly damaging to conidia. Rather, the rapid rehydration in liquid water is thought to cause lethal osmotic shock, whereas rehydration in atmospheres of high relative humidity before resuspension in water maintains conidia viability. This was not found to be the case with conidia of P. convolvulus. Rehydration at 100% RH did not improve germinability of conidia dried in water with germination being 0% with and without rehydration. Immediate rehydration at 100% RH did slightly improve germination of conidia dried in matrix (3.8% compared with 0% for conidia without rehydration), but this was almost negligible when compared with the freshly isolated conidia of the same preparation, which had 89% germination without drying. The observed 3.8% germination of conidia dried in matrix and rehydrated before germination was reduced to 0% after 7 days of storage at 21 C.
These observations indicate that conidia of *P. convolvulus* are indeed susceptible to drying. In contrast, germination of dried conidia of *Colletotrichum graminicola* (Ces.) G. W. Wils. was greatly stimulated by rehydration in 100% RH (13), whereas the conidial matrix of *C. capsici* (Syd.) E. J. Butler & Bisby did afford some protection against desiccation for conidia of this latter pathogen (7).

In contrast to dried conidia, conidia stored in either native matrix or matrix diluted 10-fold with water showed initially improved germination up to 100%, which subsequently slowly declined. These observations suggest that freshly isolated conidia of *P. convolvulus* may require a maturation period which normally occurs in the environment provided by the matrix and that the matrix retards loss of conidia viability. This would explain the variability in the germination of control conidia sometimes encountered from one batch of conidia to the next. Other similar protective roles of the conidial matrices of other fungi have been described by other workers (3,8,13).

The conidial matrix of *P. convolvulus* has little direct role in the leaf anthracnose disease of field bindweed. Occasionally, a slight stimulation or inhibition of disease was observed but was either not statistically significant or not reproducible. This was likely attributable to variability from one matrix preparation to another. Other workers suggest that the conidial matrices of other pathogens contain certain degradative enzymes that may facilitate pathogenesis (2,9,11). It appears, however, that the major functions of the matrix of *P. convolvulus* are to inhibit conidia germination and to prolong conidia viability in native matrix preparations until an ecologically more favorable dispersal has been achieved.

The work presented here is relevant to the future development of *P. convolvulus* as a mycoherbicide for field bindweed. Our observations indicate that desiccation is relatively lethal to conidia of this pathogen. Consequently, any mycoherbicidal applications should guard against such desiccation before infection of the target plant. Similarly, although not directly addressed or easily quantified, it is suggested that the conidial matrix contains a mixed population of conidia differing in such characteristics as maturity, infectivity, and survivability. Ideally, these characteristics should be maximized with the range of variability reduced. The matrix of convolvulus may serve this role in nature, and these matrix characteristics may ultimately be useful in improving conidia germinability or “shelf life” of future commercial prepartations of the mycoherbicide.

**LITERATURE CITED**


