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## Epizootiology of the Fungal Pathogen, *Zoophthora Phytonomi* (Zygomycetes: Entomophthorales) in Field Populations of Alfalfa Weevil (Coleoptera: Curculionidae) Larvae in Illinois

Marilyn J. Morris  
*University of Illinois*

Stephen J. Roberts  
*University of Illinois*

Joseph V. Maddox  
*University of Illinois*

Edward J. Armbrust  
*University of Illinois*

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EPIZOOTIOLOGY OF THE FUNGAL PATHOGEN,  
*ZOOPHTHORA PHYTONOMI* (ZYGOMYCETES: ENTOMOPHTHORALES)  
IN FIELD POPULATIONS OF ALFALFA WEEVIL  
(COLEOPTERA: CURCULIONIDAE) LARVAE IN ILLINOIS

Marilyn J. Morris, Stephen J. Roberts, Joseph V. Maddox,  
and Edward J. Armbrust<sup>1</sup>

ABSTRACT

The influence of the fungal pathogen, *Zoophthora phytonomi*, on larvae of the alfalfa weevil, *Hypera postica*, was studied in three alfalfa fields in Illinois. Disease epizootics occurred in all three fields and disease onset was observed within a fairly narrow range of degree day accumulations. At the height of each epizootic, percentages of infected larvae were between 80 and 100%, and the fungus contributed to the collapse of the weevil population in each field. Percent parasitism by the larval parasitoids, *Bathyplectes curculionis* and *B. anurus*, was lower in our fields than is common in mid-season alfalfa weevil populations and was sometimes correlated negatively with *Zoophthora phytonomi* infection levels, strongly implying negative interference between the parasitoids and the pathogen. Control potential of *Zoophthora phytonomi* disease in alfalfa weevil larval populations is addressed.

Larvae of the alfalfa weevil, *Hypera postica* (Gyllenhal), are pests of the first cutting of alfalfa in Illinois, where they can account for significant losses in crop yield and quality. Adult weevils overwinter and, depending on weather, may lay eggs during autumn, on warm days throughout winter, and during spring. Eggs hatch in spring about the time alfalfa resumes growth, and larval feeding continues through May, when pupation occurs. Upon emergence, adults leave the field for field edges where they undergo summer aestivation. During autumn, adults return to the field and begin egg-laying (Manglitz and App 1957).

Both parasitoid wasps and a fungal pathogen infect the larval stage of the weevil in Illinois. The wasps, *Bathyplectes curculionis* (Thomson) and *B. anurus* (Thomson), were released in Illinois by the USDA in the 1960s and 1970s and now are found in most alfalfa weevil populations in the state (Dysart and Puttler 1965, Dysart and Day 1976). Development of the immature parasitoid within the alfalfa weevil larva eventually kills the host.

The fungal pathogen, *Zoophthora phytonomi* (as described in Ben-Ze'ev and Kenneth 1982), infects and kills alfalfa weevil larvae; when death oc-

<sup>1</sup> Center for Economic Entomology, Illinois Natural History Survey, 607 E. Peabody, and Department of Natural Resources and Environmental Sciences, College of Agriculture, University of Illinois, Urbana-Champaign, IL 61820.

curs, one of two spore types, conidia or resting spores, is formed. Conidia released by infected larvae are responsible for horizontal spread of infection during the same season, while resting spores remain in the soil and provide the next year's inoculum (Ben-Ze'ev and Kenneth 1980). The disease was first detected in Canada in 1973 (Harcourt et al. 1974) and since then has been observed infecting alfalfa weevils in New York (Muka 1976), Missouri and Nebraska (Puttler et al. 1978), Kentucky (Nordin et al. 1983), Virginia (Los and Allen 1983), and California (Johnson et al. 1984). In Illinois, alfalfa weevil larvae infected with *Zoophthora phytonomi* were first collected in 1979 (Barney et al. 1980). Detailed epizootiological studies have been conducted in Kentucky (Nordin et al. 1983) and Virginia (Los and Allen 1983), but until our research, no in-depth study has been done in Illinois. Since earlier surveys in Illinois showed infection levels of up to 93% in alfalfa weevil larvae (Barney et al. 1980), potential interference between the disease and existing larval parasitoids, *B. curculionis* and *B. anurus*, was also a major concern.

Although this work was conducted in 1984 and 1985, this study is relevant to the development of regional pest management systems in Illinois and can be compared with similar studies in other states. The areas chosen for this study incorporated 3 fields along a ca. 130 mi. north/south transect with 65 miles between successive fields. This area includes the south central and southern portions of Illinois, which usually have economically damaging populations of weevils.

We began an epizootiological study of this disease in Illinois in 1984. Field populations of alfalfa weevils were monitored throughout the larval season (April through May in order to determine larval population levels and the extent of parasitism by *Bathyplectes* spp. and infection by *Zoophthora phytonomi* disease. The objectives of the current study were to: (1) define the relationships between disease onset and both degree-day accumulations and larval population levels, (2) measure the effect of the disease on alfalfa weevil larval populations, (3) measure the effect of the disease on the success of the parasitoids, and (4) clarify the potential of *Zoophthora phytonomi* disease as a biological control agent in alfalfa weevil populations.

## MATERIALS AND METHODS

During 1984, two alfalfa study fields were monitored: one in Dixon Springs, Pope County, in the extreme southern part of the state and the other in Mascoutah, St. Clair County, in the St. Louis area. In 1985, a new study field, located west of Casey in Cumberland County, in east central Illinois, was chosen because of its relative proximity to Champaign and because it contained a large weevil population. The research was conducted in a one-hectare plot located in the southwestern corner of each alfalfa field. Sampling was initiated when approximately 100 Celsius-degree days (Cdd), based on a 9°C developmental threshold temperature for the alfalfa weevil (Koehler and Gyrisco 1961, Litsinger and Apple 1973), had accumulated from January 1. Temperature data were obtained from weather boxes at each field location.

To address our objectives, sampling methods for estimating both disease and parasitism levels were necessary; three sampling techniques were used

over the two year period. In the two 1984 fields, two sampling methods (A and B) were used. Procedures for method A were developed by researchers involved in a three-state (Indiana, Illinois and Wisconsin) regional project. Method B was added for comparison with method A and, because of its convenience, to enable sampling an additional field. In the Mascoutah field, both sampling methods (A and B) were used and sampling was conducted weekly. In the Dixon Springs field, the simpler of the two methods (B) was employed and samples were taken at 2-3 day intervals.

In the more complicated method (A), the hectare was divided into nine equal sampling units, and a unique square foot area (.09 m<sup>2</sup>) within each unit was selected randomly on each sampling date. Twenty alfalfa weevil larvae, 10 small (1<sup>st</sup> and 2<sup>nd</sup> instar) and 10 large (3<sup>rd</sup> and 4<sup>th</sup> instar), were removed from the foliage within each square foot area for a total of 180 larvae/hectare. Post-collection procedures for these larvae are described later in this section. Population levels were determined for method A by cutting another square foot (.09 m<sup>2</sup>) area of foliage adjacent to the first within each of the nine units, and removing and counting all larvae from the foliage with the use of a modified Berlese funnel in the laboratory.

In the simpler method (B), used in both Dixon Springs and Mascoutah, five evenly-spaced sampling sites (four corner and one center locations) were located within the hectare. For determination of disease and parasitism levels, Fifty larvae (25 small and 25 large) were removed from a total of 30 stems; six stems gathered from each of the five sampling sites. The instar ranges for the large and small larvae were the same as described previously. In method B, population totals were determined by counting all larvae on the 30 cut stems, including those used to estimate disease and parasitism.

We later identified weaknesses in the 1984 sampling plan, such as overly time-consuming sampling plans (method A), inadequate sample size (method B) and infrequent sampling (Mascoutah). A new plan was developed for 1985. The 1985 plan (method C), was used at Casey, and the sampling interval was two times/week throughout the sampling season. The hectare was divided into nine equal sampling units, twelve stems were collected randomly from each unit, and all larvae on each stem were removed and examined to determine disease and parasitism levels. Population density was determined by cutting the foliage from two one-half square foot (.045 m<sup>2</sup>) areas in each unit and removing and counting all larvae as in method A.

For all methods used to determine disease and parasitism levels, after removal from the foliage, larvae were placed in individual diet cups, each with an alfalfa tip and a moist cotton wick. Every two to three days, fresh alfalfa foliage was provided and cups were checked for dead larvae. As larvae died, they were examined for disease symptoms. Wet squash mounts were prepared from each dead larva or pupa and were examined microscopically for the presence of resting spores or conidiospores. The squash mounts were not examined for developing parasitoids. Non-infected larvae were checked until they pupated, or, if they were parasitized, until wasp larvae had emerged and pupated.

At Dixon Springs, cutting was delayed and sampling continued until the weevil population had dwindled to very low numbers. At Mascoutah, sampling was terminated by the scheduled cutting only two weeks after we collected the first diseased larva and before the epizootic had much time to develop. In 1985, the Casey study field was not cut until the weevil population was very low and sampling had been terminated.

## RESULTS AND DISCUSSION

## Epizootic phenology

Disease onset was shown to be strongly associated with a specific range of degree-day accumulations. For this discussion, we are equating first detection of disease with disease onset and the initial stages of an epizootic. In the Illinois fields, the first diseased larvae were detected when Cdd accumulations were between 150 and 220. The Cdd accumulations for alfalfa weevil larval development (Hsieh et al. 1974) for first through fourth instar are 70, 38, 41 and 61 Cdd respectively. Interstate values from Illinois, Kentucky, Virginia, Indiana, and Wisconsin range from 150 in south central Illinois to 287 in Kentucky, with a median value of 220 and a mean of 224 (Table 1; Los and Allen 1983, Nordin et al. 1983; Wisconsin and Indiana unpublished data; Indiana, Illinois, Wisconsin regional project 1984–85. Eighty percent of all values are within 50 Cdd of the mean. An average of 13 years of Cdd accumulations in Ontario, Canada (Harcourt et al. 1990) yielded a mean of 204 Cdd. In a study in Iowa from 1990–92 Giles et al. (1994) determined a mean of 235. These values are very similar, especially considering the extensive range of latitudes and locations represented, and that 10 to 15 Cdd per day can accumulate during the spring. The consistency of these values underscores the usefulness of degree-day accumulations for predicting disease onset.

To analyze the effect of the disease epizootic on the alfalfa weevil population and on parasitoid activity, larval numbers, infection levels, and parasitism levels were compared for each field (Fig. 1, Fig. 2, Fig. 3). Correlations (Spearman's) between population density and percent infection were calculated for the Dixon Springs and Casey fields where cutting was delayed and the epizootic could be monitored for its entire duration. For both fields, analyzing full-season data yielded significant negative correlations between population levels and percent infection (Table 2). Dividing the sampling period into two phases helped to clarify seasonal patterns. In the Dixon Springs and Casey fields, weevil population levels initially increased and continued sharply upward until infection levels approached 30%. After that time, alfalfa weevil larval population levels began to fall sharply, usually over a period of about 10 days. Throughout this period, infection levels continued to increase, reaching between 80 to 100% by the end of the season. In the early part of the season, both the weevil population and infection levels were increasing and were significantly positively correlated, but by late season, decreasing population and increasing infection levels produced negative correlations. The weevil population continued to decline as a result of the fungal pathogen and pupation of 4<sup>th</sup> instar larvae. Ultimately, there were so few larvae present in fields that sampling was discontinued.

## Disease/parasitoid interaction

In addition to measuring the effect that the *Zoophthora phytonomi* disease exerted on the alfalfa weevil population, we examined its effect on the success of the established parasitoids. Since both the pathogen and the parasitoids affect the larval stage of the weevil, compatibility among these biological control agents may in part determine their individual success. *Bathyplectes curculionis* and *B. anurus* were first detected parasitizing alfalfa weevil larvae in Illinois fields in 1964 (Dysart and Puttler 1965) and commonly have been responsible for parasitism levels of 30 to 40% in the state. *Bathyplectes anurus* is most active early in the weevil season, but parasitism by *B. curculionis* typically builds throughout the larval season and peak par-

Table 1. Observed calendar date, cdd accumulations, % infection and population density at first incidence of *Zoophthora phytonomi* disease for alfalfa fields in Illinois, Kentucky, Virginia, Indiana and Wisconsin.

Year, location and sampling method	Calendar date of disease onset	Cdd at disease onset	% infection	No. larvae/stem
<b>ILLINOIS</b>				
1984				
Mascoutah A	3 May	150	1.4	1.9
Mascoutah B	9 May	174	3.3	1.6
Dixon Springs B	30 April	220	2.2	4.7
1985				
Casey C	23 April	194	.8	.76
<b>KENTUCKY</b> <sup>a</sup>				
Spindletop				
1980	10 May	222	4.5	—
1981	2 April	270	<1	—
1982	5 May	274	<1	—
Glasgow				
1980	1 May	234	1.7	—
1981	14 April	249	2.7	—
1982	29 April	287	10.0	—
<b>VIRGINIA</b> <sup>b</sup>				
1979	8 May	246	49.0	—
1980	23 April	200	.4	—
1981	15 April	195	2.3	—
<b>INDIANA</b> <sup>c</sup>				
1984				
Bedford	8 May	286	2.5	—
<b>WISCONSIN</b> <sup>c</sup>				
1984				
Dane Co.	4 June	199	.5	—
Door Co.	6 June	186	2.5	—

<sup>a</sup> Data from Nordin et al. 1983<sup>b</sup> Data from Los and Allen 1983<sup>c</sup> Unpublished data

asitism follows the larval population peak in early to mid May (Richardson et al. 1971, Puttler 1967, Miller et al. 1972, Miller and White 1973).

*Bathyplectes curculionis* was overwhelmingly the most prevalent of the two species in all study fields, constituting between 80 to 100% of wasp cocoons produced. In the two fields where delayed cutting allowed extended sampling (Casey and Dixon Springs), parasitism levels were at their highest at the beginning of the weevil season and detection of parasitized larvae pre-

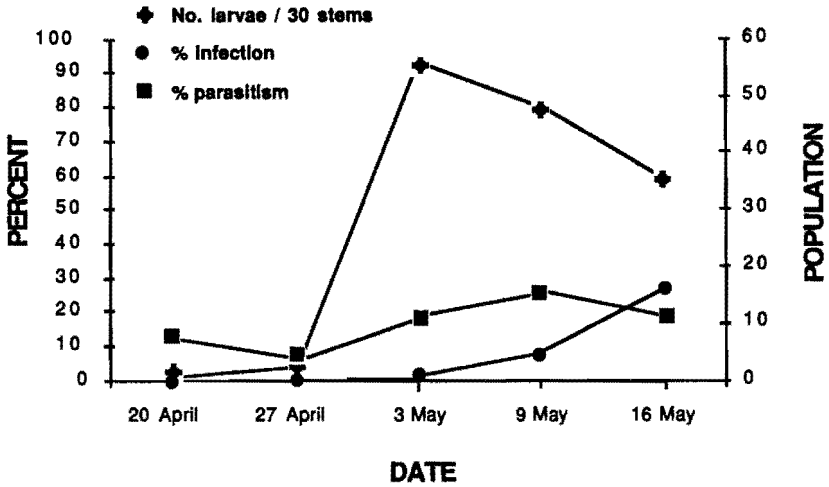


Figure 1. Alfalfa weevil larval population, % infection and % parasitism at Mascoutah, Illinois, 1984.

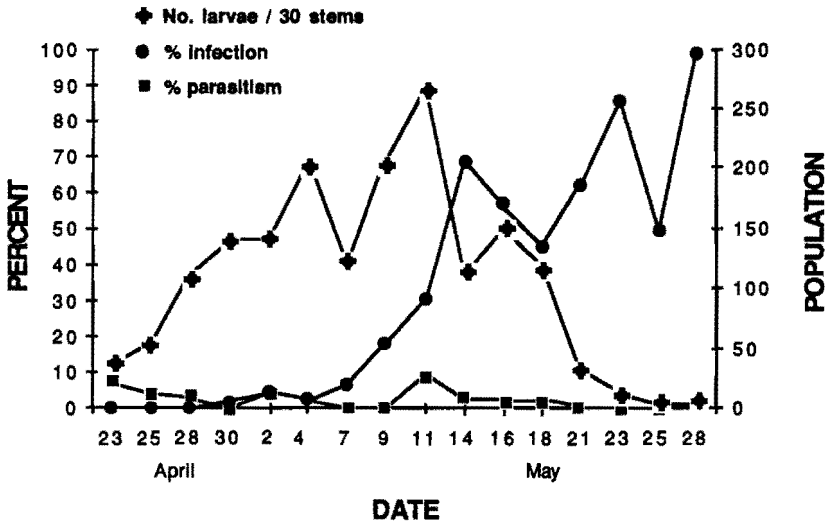


Figure 2. Alfalfa weevil larval population, % infection and % parasitism at Dixon Springs, Illinois, 1984.

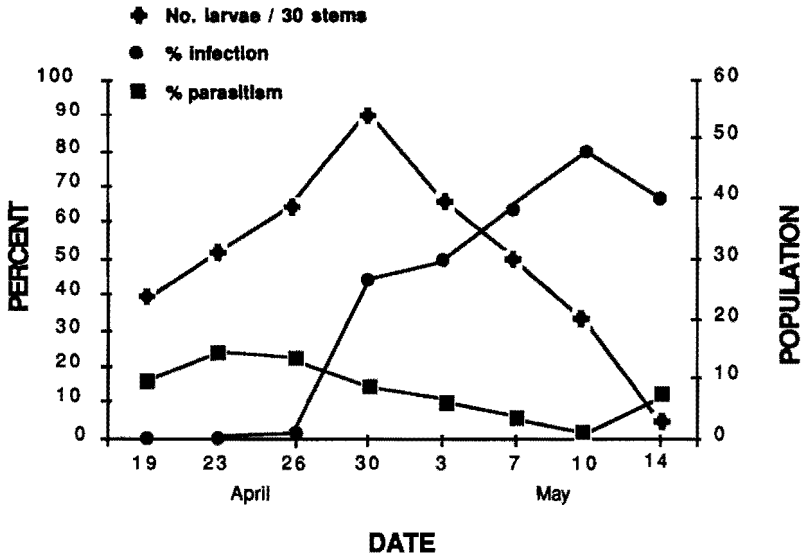


Figure 3. Alfalfa weevil larval population, % infection and % parasitism at Casey, Illinois, 1985.

ceded onset of disease by approximately two weeks (Fig. 2, Fig. 3). Parasitism decreased as larval counts increased. During the first part of the season, when infection levels were low, correlations between percent parasitism and percent infection were not significant. During the latter part of the season, when infection levels were at their highest, percent infection and parasitism always were significantly negatively correlated, with parasitism falling while infection rose (Table 2). In the 1984 Mascoutah field, a cutting interrupted sampling, but a trend similar to that noted in the other two locations may have been developing (Fig. 1). From 27 April through 9 May, both parasitism and percent infection were increasing, with parasitism about 15% higher than infection. On the last sampling date, (16 May), however, parasitism levels had fallen while infection levels had increased, so that infection was more prevalent than parasitism.

Two biocontrol agents attacking the same stage of the host have the potential of negatively affecting each other. Interference between fungal pathogens and parasitoids has been previously documented (King and Bell 1978, Loan 1981). In the alfalfa weevil, because of the difference in length of time from infection or parasitism until death (approximately five days for the fungus and 10 days, or until weevil pupation, for the parasitoid), a larva infected and parasitized simultaneously most likely would die from the disease before the parasitoid could complete its development within the diseased host. This competition would vary depending on the relative times of infection and parasitism. These effects, however, have never been examined for this particular insect/host/parasitoid/pathogen relationship. Our field data support the conclusions of Los and Allen (1983) that interference does occur among the parasitoids and the pathogen, and that the parasitoids are af-



Table 2. Correlation coefficients of the relationships between percent *Zoopthora phytonomi* infection and population density of *Hypera postica* and between percent *Zoopthora phytonomi* infection and percent parasitism by *Bathyplectes* sp. in Illinois. (n = no. larvae examined for infection and parasitism)

Year and location	<i>Zoopthora</i> sp. correlation coefficient	and <i>H. postica</i> level of significance	<i>Zoopthora</i> sp. correlation coefficient	and <i>Bathyplectes</i> spp. level of significance
<u>1984: Dixon Springs</u>				
all season (n=534)	-.450	.05>p>.025	-.361	NS .1>p>.05
4/23-5/9 (n=321)	.622	.05>p>.025	-.547	NS .1>p>.05
5/9-5/28 (n=213)	-.713	.025>p>.01	-.682	.05>p>.025
<u>1985 Casey</u>				
all season (n=890)	-.682	.025>p>.01	-.718	.025>p>.01
4/16-4/26 (n=364)	.978	.001>p>.005	.687	NS .25>p>.10
4/30-5/14 (n=526)	-.819	.05>p>.025	-.859	.025>p>.01

ected negatively by the presence of the pathogen. Loan (1981), in Ontario, Canada, used a fungicide to control *Zoophthora phytonomi* disease in half of his study plots and was able to reduce the incidence of diseased larvae by 88 to 99%. He found that in untreated plots 13% of the alfalfa weevil larvae were parasitized by larval parasitoids, *Microctonus colesi* Drea, while in treated plots 77% of larvae were. Results of Loan (1981), strongly support conclusions of Los and Allen (1983) and of the 1984–85 Illinois data presented herein. It seems likely that if the pathogen remains active in Illinois, the parasitoid population eventually will decline significantly.

#### Control potential of *Zoophthora phytonomi* disease

Documented accounts of the progression of disease epizootics in specific locations show that control from the disease varies considerably with location. In Ontario, Canada, based on a ten-year study, Harcourt et al. (1984) concluded that the alfalfa weevil has ceased to be an economic problem due to the high level of infection from *Zoophthora* sp. disease. Epizootics there occurred early enough in the spring to control the pest. In Virginia (Los and Allen 1983) and Kentucky (Nordin et al. 1985) and in previous years in Illinois, epizootics have usually occurred too late in the spring to exert adequate control. In the present study, however, we found variation among study sites regarding control potential. We believe that the epizootic in the Casey field in 1985 was directly responsible for preventing the larval population from reaching the economic threshold, but this was not the case in the other two study fields.

Development of an epizootic and success of *Zoophthora phytonomi* disease as a control agent strongly depends on the temporal relationship among fungal, insect, and crop growth. These relationships are, therefore, ultimately dependent on climatic influences. Climatic conditions (temperature, humidity, rainfall) affect each component in the alfalfa ecosystem differently.

In addition, certain characteristics of an insect host population may influence the ability of a pathogen to maintain the population at sub-economic levels. The size of population peaks and the temporal spacing between them may be important. Thorvilson and Pedigo (1984), studying the epizootiology of *Nomuraea rileyi* in soybeans, found that the relative size of first and second generations of green cloverworm larvae strongly affected the development and extent of fungal epizootics. While alfalfa weevil larvae are predominantly univoltine, separate population peaks, dependent on the relative number of fall-, winter-, or spring-laid eggs, may be visible within that generation. In areas where substantial fall and/or winter egg laying occurs, a large first wave of larvae hatches in early spring. If this population exceeds the disease threshold, the epizootic may begin at this time. The already initiated epizootic will cause the infection to spread rapidly through larvae hatching from spring-laid eggs and the population may be controlled. This phenomena was observed in the Casey field in 1985, when well into the season, a second wave of larvae hatched and subsequently experienced high fungal-induced mortality. Once the epizootic is initiated, and the weather remains relatively cool and wet, this fungal pathogen will continue infecting larvae until the population is reduced to extremely low levels. In areas or seasons where spring egg-laying substantially exceeds fall and winter egg-laying, the onset of an epizootic will most likely develop from larvae that hatched from spring laid eggs rather than from eggs laid in fall. In most years, the fungal pathogen doesn't become epizootic early enough in Illinois to prevent weevil larvae from reaching the economic threshold.

Cultural control practices may be manipulated to aid the development of a fungal disease already present and active in the field. At Dixon Springs in 1984, the alfalfa crop grew quickly in the cool temperatures and harvest could have been performed before damage was severe. Our delayed cutting for research purposes prolonged larval feeding and allowed more crop damage, an atypical situation. An early harvest in fields where larval populations are high and disease activity is present will halt damage to the first crop and may encourage an epizootic in larvae feeding on the second crop. After the alfalfa foliage is removed, larvae are exposed to the soil containing fungal inoculum, which could initiate additional infection and possibly promote an epizootic (Johnson et al. 1984).

*Zoophthora phytonomi* disease has been shown to have high potential as a naturally occurring biological control in a variety of situations. The exact requirements necessary for the development of the disease are still unknown. Further study is necessary before *Zoophthora phytonomi* becomes a consistently useful component of an alfalfa weevil integrated pest management program. In addition, the long-term effects of the fungus on established parasitoids merits continued study. These two areas of research could be explored through both field and laboratory studies. Finally, incorporation of *Zoophthora phytonomi* disease into control programs depends largely on the development of accurate and efficient sampling methods.

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