

STUDIES ON RED CROWN ROT OF SOYBEAN: INCIDENCE IN ILLINOIS, FUNGICIDE  
SEED TREATMENTS, AND HOST RESISTANCE

BY

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THESIS

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## ABSTRACT

Soybean [*Glycine max* (L.) Merr.] is an economically important crop in Illinois, and also globally. In 2021, there was an estimated 4.28 million hectares of soybeans planted in the state of Illinois. *Calonectria ilicicola* Boedijn & Reiltsma is a soilborne fungus that causes red crown rot (RCR) of soybean. The pathogen was originally described causing a disease in peanuts, called Cylindrocladium black rot, in Georgia in 1965. RCR was first reported on soybean in the United States in 1972. This fungus that causes RCR also causes pre- and post-emergence damping off in soybean as well as root rot and foliar chlorosis and necrosis after flowering. Infected plants die prematurely reducing seed production.

There were 14 confirmed counties with RCR in Illinois including Bureau, Christian, Effingham, Fayette, Ford, Kankakee, Livingston, Madison, Marion, Pike, Tazewell, Sangamon, St. Clair, and Woodford based on 2020 and 2021 field surveys in southern and western Illinois and based on the University of Illinois Plant Clinic data of positive samples of RCR from 2018 to 2021. Based on locations of these 14 counties, RCR encompasses a wider distribution in Illinois than just the southern or western part of the state.

Literature indicates that rotation between grain crops (non-legume), avoiding or reducing wet areas in the field, and tillage might be effective for RCR management. Soybean fungicide seed treatment labels do not include RCR, which led to several experiments to determine if fungicides are effective in controlling seed rot, pre- and post-emergence damping off caused by *C. ilicicola*. Overall, CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> + Saltro<sup>®</sup> provided the best treatment results that were equal to the non-inoculated control for stand counts, root rot ratings, and plant weights.

Another approach that has not been well explored for the potential management of red crown rot is the use of host plant resistance. There is very little information about screening soybeans to identify sources of resistance to this pathogen, *C. ilicicola*, and to date, no cultivars have been developed with resistance to RCR. Three experiments were completed, one for breeding lines/cultivars and two for soybean ancestral lines. For the breeding lines/cultivar experiment, LD10-10219 had the lowest root rot rating. In the first ancestral experiment, which included older modern cultivars among the ancestors, Corsoy 79 and Williams 82 generally had the lowest root rot ratings. In the second ancestral experiment, Mukden, Tokyo, PI 54610, and Dunfield had the lowest root rot ratings. The significant differences among genotypes observed in these evaluations show that some genotypes may carry genes for resistance to *C. ilicicola*.

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## CHAPTER 1: LITERATURE REVIEW

### Introduction

Red crown rot (RCR) of soybean is caused by *Calonectria ilicicola* Boedijn et Reltsma, a soilborne fungus. The pathogen was originally found on peanut in Georgia in 1965 (Bell and Sobers 1966) and first reported on soybean in the United States in 1972 (Kim et al. 1998). In soybean this fungus causes pre- and post-emergence damping off as well as root rot and interveinal foliar chlorosis and necrosis after flowering. Infected plants die prematurely reducing seed production and seed weight (Akamatsu et al. 2020).

Soybean is an economically important crop in the United States and globally as it is an important source of protein and oil and generates income through domestic use and exports. In the United States, the cash value of soybean exports was \$US 46.1 billion with China, Mexico, Egypt, Netherlands, and Japan as the five top importers (NASS 2022). In general, soybean production in the United States has been increasing and 2020 brought in record yields (3.4 tons per ha or 51 bu /acre; NASS 2022). In 2020, Illinois was the number one state for soybean production with 16 million metric tons (604 million bushels; NASS 2022).

RCR has been reported in southern states in the United States (Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Virginia), as well as Australia and parts of Asia (Padgett et al. 2015) and more recently in Illinois (Kleczewski et al. 2019) and Kentucky (Blake 2021). In China, the first case of RCR was reported in 2002 with some fields having a disease incidence of up to 80% (Gia et al. 2017). In Japan, the disease is geographically widespread, but within a given field it was reported to occur mainly in patches (Yamamoto et al. 2017).

There are a number of hosts that *C. ilicicola* impacts that will be discussed later in this review, but the main one in the United States is peanut and the spread of *C. ilicicola* is believed to have followed peanut production throughout the southeast (Allen 2013). In China, the rotation of peanut and soybean was believed to cause an increase of RCR in soybean (Gia et al. 2017). In Illinois, RCR was first observed in a commercial soybean field in Pike County in 2018 and confirmed in 2019 to be RCR with a field incidence estimated at 75% (Kleczewski et al. 2019). In 2020, plant samples from 27 different fields from southern and western Illinois were examined resulting in 10 fields in four counties confirmed to contain *C. ilicicola* (Chapter two). Although none of the fields had a known association with peanut production, the pathogen may have been brought on equipment from peanut growing areas. However, there are other possibilities besides contaminated equipment to explain how the fungus got started and spread in Illinois.

### **Signs and symptoms of red crown rot of soybean**

The genus of *Calonectria*, first described in 1867, belongs to the Phylum Ascomycota, Class Sordariomycetes, Order Hypocreales. The genus is characterized by production of yellow to orange to red perithecia. Sexual reproduction of members of this genus occur by homothallic (i.e. *C. ilicicola*) or heterothallic mating systems, and some species have both (Lombard et al. 2010). Many of the species in the genus, like *C. ilicicola*, produce both asexual and sexual spores. The functionality of spores produced by *C. ilicicola* needs further investigation, but presumably they play a role in dispersal and infection (Akamatsu et al. 2020).

*C. ilicicola* produces burnt red perithecia that are 300-500 µm in height and 280-400 µm wide (Bell et al. 1966). The perithecia are round and contain clavate asci that are narrow at the base and thick at the apex. Each ascus has four or eight curved ascospores with 1-3 septae each.

The conidia of *C. ilicicola* are cylindrical and measure 38-68  $\mu\text{m}$  x 4-5  $\mu\text{m}$  . The conidia are produced by apical budding and have 1-3 septae (Bell et al. 1966).

Mature ascospores can be observed within two to three weeks after infection, and they are a responsible for the secondary disease spread in fields (Bell et al. 1966). Conidia are rarely found in field conditions. The ascospores mature under high relative humidity (100% night-time humidity) and spread by ejection and rain splashing. In the USDA-ARS Fungal Database, 186 records of *C. ilicicola* and its synonyms have been recorded from many legume and non-legume hosts (Farr and Rossman 2021).

Foliar symptoms of RCR are most noticeable when soybean plants are past flowering, but infections may occur soon after planting seeds as the fungus can cause seedling damping off and early root rot. Later in the season, infected plants will have a reddish discoloration with bright colored perithecia embedded in the epidermal tissue on the outside of the lower stem at the crown of the plant. Roots will be rotted, and plants easily pulled from the soil. RCR appears more frequently in warm, humid, and moist environments.

The foliar symptom phase of RCR on soybean appears to be similar to sudden death syndrome (SDS) in that the fungus infects and colonizes roots and a toxin translocates through the vascular system to the leaves causing foliar symptoms (Yamamoto et al. 2017). Interveinal leaf tissue turns yellow and brown from the bottom leaves to the top. Unlike SDS, the leaves will not prematurely defoliate. The inside of the stem will have a gray discoloration (Yamamoto et al. 2017). From a distance, the foliar symptoms make the disease indistinguishable from SDS.

Due to the similarity of some of the disease symptoms to RCR, molecular confirmation of the fungus in soil and in plants has been reported (Ochi and Kuroda 2020). RCR impacts Japan's soybean production, and to understand the fields RCR incidence before PCR, researchers

would wet sieve the soil. The process of wet sieving was time consuming timely, so qPCR increased the efficiency and allowed for more samples to be processed (Ochi and Kuroda 2020). The molecular method uses the loop-mediated isothermal amplification to target the  $\beta$ -tubulin gene region to detect *C. ilicicola* (Ochi and Kuroda 2020).

### **Other hosts**

**Peanuts.** There is more information about *C. ilicicola*, and the disease known as *Cylindrocladium* black rot of peanuts than what has been reported on soybeans. On peanuts, the disease occurs more commonly in soils that are poorly drained (Anco and Chapin 2016). Symptoms include leaf chlorosis and taproot rot; signs of the fungus include bright colored perithecia on stem, pegs, pods, and roots of the plant (Gia et al. 2017). Yield losses of over 50% have been reported on peanut (Gia et al. 2017). The fungus can infect peanut seed and be seed-transmitted (Ochi et al. 2011). Symptoms of *Cylindrocladium* black rot are often visible on the hypocotyls that turn yellow and die, and on tap roots which become black and rotted. (Ochi et al. 2011).

On soybean the disease cycle for RCR is assumed to be the same or similar to the disease cycle for *Cylindrocladium* black rot of peanuts (Allen 2013). For *Cylindrocladium* black rot, the microsclerotia overwinter and are the long-term survival units that reside in the soil often associated with plant debris. Equipment and wind can help spread the microsclerotia throughout the field or to other fields. Once soil temperatures are 23-25°C, the microsclerotia germinate and enter the root tip of peanuts by penetrating the cortex. As the disease develops, the fungus will produce perithecia on infected roots and the base of the stem. Ascospores will disperse when perithecia are mature. *Cylindrocladium* black rot can infect peanut seed which increases the risk of seedborne spread (Gai et al. 2012).

**Blueberries.** *C. ilicicola* is considered one of the main pathogens in blueberry nursery stock in both Georgia and North Carolina (Haralson et al. 2021). *C. ilicicola* causes severe blight and death of blueberry seedlings. The course of the disease starts with infection through wounding, leading to browning of the stem and wilting of the leaves. Around one week after infection, seedlings start to die off. Orange to red perithecia will develop around in the infected tissue. The fungus has not been found in mature blueberry bushes, but it has been found in neighboring peanut fields. The fungus has been isolated from blueberry seedlings, and both blueberries and peanuts cultivars are susceptible to *C. ilicicola*.

In 2007 and 2008, a survey was conducted in 18 blueberry nurseries to establish what soilborne pathogens are common in commercial blueberry propagation (Haralson et al. 2021). The leading pathogen was *C. ilicicola* in this survey. With the survey results, the most common pathogens in blueberries were noted for future research studies, along with the researchers communicating to nursery managers on ways to improve their management practices to reduce the level of disease in commercial blueberry nurseries (Haralson et al. 2021).

**Boxwood.** Boxwood blight is a destructive disease in the landscape industry. The casual pathogen(s) causing boxwood blight is *Calonectria henricotiae* and *C. pseudonaviculata*. This disease was first found in the United Kingdom in 1994 and can now be found worldwide. Currently, there is no cure for boxwood blight, but fungicide use can reduce, or slow disease development and host resistance should be used if available (Yang et al. 2021).

**Additional hosts.** As mentioned earlier, there are many hosts that *C. ilicicola* and associated species infect. Besides the hosts mentioned above, there are a few others of interest and worth mentioning. One is rose, where *C. cylindrospora* causes *Cylindrocladium* crown rot. This disease was thought to be eradicated from rose nurseries in Florida 26 years ago, but it was

recently found again in a Florida nursery in 2017 and immediately eradicated (bushes destroyed) and has not been reported since (Iriarte et al. 2021). Another recent report from southern China found *C. pentaseptata* causing leaf blight on *Eucalyptus* leaves in commercial plantations (Wang and Chen 2020). *C. ilicicola* has been found and confirmed on several weeds including partridge pea, sickle pod, and beggarweed (Bell et al. 1966), and all of these occur in Illinois (Loux et al. 2020).

### **Management of RCR in soybean**

Management of RCR relies on cultural practices that include rotation with rice or other grain crops, fallow periods to decrease inoculum, avoiding planting during wet periods, and tillage (Akamatsu et al. 2020). One of the most effective management options is crop rotation by planting corn or another nonlegume for a few years as this can greatly decrease the viable microsclerotia in the soil (Jiang et al. 2020). However, because the pathogen infects many hosts and microsclerotia have a long-life span, the disease is still difficult to manage (Jiang et al. 2020).

Besides rotation there are other potential management options. In Japan, inoculating soil with rhizobia and mycorrhizal fungi decreased disease presence through their association with the plant (feeding and protecting the plant) limiting *C. ilicicola* entry into the plant through the nodules (Akamatsu et al. 2020).

Another possible management option is through the use of fungicide seed treatments. The control of diseases with fungicides has a long history that goes back to the 17<sup>th</sup> century when wheat grain was brined with salt water followed with liming the seeds to control bunt in harvested seed (Morton and Stuaab 2008). Another technique to control bunt was to soak seeds in purified urine and a copper solution, and although the urine was not very effective, the copper

solution was and became popular until the 20<sup>th</sup> century when conventional fungicides became popular (Klittich 2008). In 1882, Millardet noticed that grape vines sprayed with copper sulfate and lime mixture did not defoliate like leaves on unsprayed vines (Morton and Staub 2008). In 1913 in Germany chloro (2- hydroxyphenyl) mercury was introduced to cereals (Klittich 2008). In the 1940s, copper sulfates and mercury were used for horticulture crops from seed treatments to foliar sprays (Morton and Staub 2008). After the 1940's, new fungicide chemistries were introduced including the dithiocarbamates and phthalimides, inorganic fungicides, that were harder to prepare but more active against fungi (Morton and Staub 2008). Research and development grew rapidly after 1960s with the first systemic fungicide seed treatment (Morton and Staub 2008) developed in 1969 and replaced the organomercurial seed treatments in cereals (Klittich 2008).

Today, more than 80% of the fruits and vegetables grown in the United States receive fungicide treatments every season, which has been estimated to increase agricultural industry income by \$13 billion annually (Morton and Staub 2008). Seed treatments include fungicides, insecticides, and rodenticides, but fungicides are the most common treatment (Ayesha et al. 2021). Systemic fungicide seed treatments are the recommend practice to protect crops from seed and soil-borne disease (Ayesha et al. 2021). For organic farming, copper and sulfur are used as fungicides (Morton and Staub 2008).

Every spring, soybean farmers face seedling diseases that can cause economic losses in yield or costs to replant the crop (Giesler and Miller 2017). The two main types of fungicide seed coat treatments used are a contact fungicide, which is used as a protectant on the seed surface or a systematic fungicide seed treatment that is absorbed by the seedling to fight or kill the pathogen inside the plant and this type of treatment has a longer residual than contact fungicides

(Giesler and Miller 2017). Common soybean seed treatment includes, fludioxonil (Veltyma) and thiram, both contact fungicides, and azoxystrobin (Miravis Neo), mefenoxam (Apron XL), metalaxyl, trifloxystrobin (Priaxor Xemium), and thiabendazole (ILEVO), which are all systemic fungicides (Giesler and Miller 2017).

Although there are many seed treatments labeled for use on soybean, there are none that include RCR. The only literature I could find on this subject was recently published and it reported that seed treated with ILEVO<sup>®</sup> or CruiserMaxx<sup>®</sup>+ Saltro<sup>®</sup> reduced the percent of root rot in seedling tests conducted under controlled conditions (Kleczewski and Geisler 2022; see chapter 3 for active ingredient name). Further work is needed to determine if fungicides are effective in controlling seed rot, pre- and post-emergence damping off, and the late season foliar symptoms caused by this disease.

Another approach to manage RCR that has not been well explored is the use of host plant resistance. Soybean resistance could be a good economic and environmental management strategy if sources of resistance can be identified and incorporated into elite breeding stock. Previous testing of soybean cultivars for RCR resistance has so far been unsuccessful (Akamatsu et al. 2020). In Japan, the cultivars Enrei, Fukuyutaka, Jack (PI 540556), and Peking, were tested and all were found to be susceptible to the fungus (Jiang et al. 2020). In the same study, wild soybean (*G. soja*) accessions Gs-7 (JP30157), Gs-9 (JP30159), and GS-27 (JP36084) showed resistance. Although there has not been much evaluation for resistance to *C. ilicicola* in soybean, there have been several reports in peanuts including a set of 125 breeding lines that resulted in cultivar NC 12C (Hollowell et al. 2008). More work is needed in identify and breed resistance into elite breeding stock in soybean to provide cultivars with RCR resistance to farmers.

## Objectives

Literature and research related to RCR is rather sparse, and there is a need for additional studies in areas of epidemiology and disease management. The objectives of this thesis were to i.) survey for the presences of RCR on soybean in Illinois, ii.) determine the efficacy of fungicide seed treatments for control of RCR, and iii.) identify potential of sources of soybean resistance to *C. ilicicola*.

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## CHAPTER 2: OCCURRENCE OF SOYBEAN RED CROWN ROT IN ILLINOIS

### Abstract

Red crown rot (RCR) of soybean [*Glycine max* (L.) Merr.] is caused by *Calonectria ilicicola* Boedijn & Reiltsma, a soilborne fungus. Red crown rot has been reported in many soybean-producing states throughout the United States, as well as in Asia and Australia. This chapter summarizes the occurrence of RCR in Illinois based on infected soybean samples collected from farmers, collected in field surveys, and positive samples recorded from diseased plants submitted and diagnosed by the University of Illinois Plant Disease Clinic. RCR was first observed in a commercial soybean field in Pike County in 2018 and reported in 2019 after confirmation that the disease was caused by *C. ilicicola*. In addition, the University of Illinois Plant Clinic received a soybean sample that was confirmed as RCR from Christian County in 2018. There were no new reports of RCR from field surveys or from samples sent to the University of Illinois Plant Clinic in 2019. In 2020 and 2021, a survey of counties in southern and western Illinois was organized and the University of Illinois Plant Clinic provided their counts of positive samples of RCR. From these resources, 14 counties that encompass a wide distribution throughout the state of Illinois have been confirmed with RCR.

### Introduction

Soybean is an economically important crop in the United States and globally as it provides protein and oil and income through domestic use and exports. The cash value of soybean exports from the United States was \$46.1 billion with China, Mexico, Egypt, Netherlands, and Japan as the five top importers (NASS 2022). In general, soybean production in the United States has been increasing and 2020 brought in record yields (3.4 tons per ha or 51 bu/acre; NASS 2022). In 2020, Illinois was the number one state for soybean production with 16

million metric tons (604 million bushels; NASS 2022). In 2021, record high soybean production in the United States was recorded with yields up 5% from 2020 and with 21 states reaching record high yields with an average 3.4 tons per ha (51.4 bu /acre; NASS 2022).

Red crown rot (RCR) of soybean was reported in Asia, Australia, and most southern and southeastern states in the United States (Padgett et al. 2015). In the United States, RCR was first reported in Georgia in 1965 (Gia et al. 2017). In China, the first case of RCR was reported in 2002 with some fields having a disease incidence of up to 80% (Gia et al. 2017). In Japan, the disease is geographically widespread, but within a given field it was reported to occur mainly in patches (Yamamoto et al. 2017).

In Illinois, RCR was first reported in a commercial soybean field in 2019 (Kleczewski et al. 2019). Besides Illinois, RCR recently was reported in Kentucky in 2021 (Blake 2021), but the disease not been reported in states of Iowa, Indiana, Missouri, or Wisconsin that border Illinois (Fig. 2.1). Although not officially reported in Tennessee, there are reports by researchers of the disease in experiment plots (Heather Kelly, pers. comm., 2021). In Missouri, RCR is listed in the Missouri Field Guide as an emerging disease (Bissonnette et al. 2021), but after further communication it appears that it has not yet been officially reported (Kaitlyn Bissonnette, pers. comm., 2021).

Surveys for RCR mostly rely on the foliar symptoms that occur on plants at the pod-filling stages. Plants become infected before foliar symptoms appear and there is even a pre- and post-emergence damping off that occur soon after seed are planted. These early symptoms and the root rot phase of the disease are difficult to distinguish from other pathogens causing these same symptoms. Late in the season, the outside of the lower stem will have a reddish discoloration with bright red perithecia at or near the crown of the plant (Fig. 2.2). The

combination of these symptoms and signs are usually sufficient for a diagnosis. These plants will have rotted roots and plants are easily pulled from the soil. RCR appears more frequently in warm, humid, and moist environments (Padgett et al. 2015).

The foliar symptom phase of RCR is similar to the symptoms of sudden death syndrome (SDS) in that the fungus infects and colonizes roots and produces a toxin that moves in the vascular system to the leaves causing foliar symptoms (Yamamoto et al. 2017). For RCR, the leaves turn yellow starting from the older leaves to the top of the plant similar to the interveinal chlorosis caused by SDS (Yamamoto et al. 2017) but unlike SDS, the plant will not prematurely defoliate. The pith of the stem will have a gray discoloration (Yamamoto et al. 2017). From a distance, the disease may look like SDS or other diseases that cause interveinal chlorosis and necrosis.

The fungus that causes RCR also attacks a number of different plants including peanuts, blueberries, roses, and several weeds. In the USDA-ARS Fungal Database, 186 records of *C. ilicicola* and its synonyms have been recorded from many legume and non-legume hosts (Farr and Rossman 2021). *C. ilicicola* has been found and confirmed on several weeds including partridge pea, sickle pod, and beggarweed (Bell et al. 1966), and all of these occur in Illinois (Loux et al. 2020).

There have been a few surveys done to determine the distribution of RCR in a region. In Mississippi, RCR was observed in soybean prior to 2013, but the distribution of the disease was reported then to be extremely limited in the state, and after 2013, there were six counties confirmed counties in Mississippi to have RCR in soybean (Allen 2013). The first occurrence of RCR in Mississippi can be tracked back to farm equipment purchased in Louisiana (Bissonnette et al. 2021). In Texas, *C. ilicicola* was isolated from an irrigated peanut field in 2004 (Wheeler

and Black 2005). This finding of *Cylindrocladium* black rot (CBR) changed peanut seed production practices in western Texas and eastern New Mexico that included avoiding fields with confirmed cases of *C. ilicicola* and using disease free peanut seed in noninfected fields (Wheeler and Black 2005). In China, a country-wide field survey from 2008 to 2014 noted three provinces in southern China to have CBR and RCR (Gai et al. 2017).

In Illinois, RCR was found in one field in Pike County, Illinois in 2018 (Kleczewski et al. 2019). Also in 2018, RCR was confirmed in Christian County by the University of Illinois Plant Clinic. With this new disease in the state, there is a need to understand and know the distribution of this disease throughout the state. The objective of this chapter was to detail the occurrence of RCR in Illinois obtained from field surveys and positive samples from the University of Illinois Plant Clinic.

## **Materials and methods**

**Survey samples.** In 2020 and 2021, I sent a request to 28 different growers and agronomists across Illinois based on calls made to co-ops asking if they were interested in assisting with the RCR survey by collecting soybean plant samples that they believed were RCR. Each field sample sent into the lab included four to five plants, with its GPS location, field crop history, and field incidence. Samples were placed in zip lock bags, refrigerated until shipped, and then shipped overnight. Once samples were confirmed positive, the results were forwarded to participants.

In addition to the request for samples in 2021, seven fields located close to fields confirmed for RCR in 2020 were evaluated for RCR. These fields were identified based on the GPS coordinates of the 2020 positive fields. In addition, I met with an agronomist (Robert

Bellm) in southern Illinois to observe fields with RCR, noting the incidence of RCR, and tallying the number of positive fields in the eight counties visited.

**Plant Clinic confirmations from the University of Illinois.** I communicated with Diane Plewa from the University of Illinois Plant Clinic asking if the Plant Clinic diagnosed any RCR samples in the 2021 season. The diagnosis was done in the clinic by visually confirming the presence of red perithecia on the base of the stems.

## **Results**

**Survey samples for RCR in 2020.** Four surveyed counties had fields identified with RCR. A total of 27 soybean field samples were received based on the request to growers and agronomist to send in samples. Of these, ten samples were visually confirmed to be positive for RCR which included samples from Madison (three out of five fields positive), Pike (five out of eight fields positive), Sangamon (one out of four fields positive), and St. Clair (one of one field positive; Fig. 2.3) counties. There were two samples from unidentified counties. No RCR was identified in samples from Douglas (three fields), Champaign (three fields), or Macoupin (five fields) Counties.

**Survey samples for RCR in 2021.** The 2021 survey found seven counties positive for RCR. There were eight fields visually confirmed positive with RCR from Madison (six positive fields), Pike (one positive field), and Sangamon (one positive field) Counties. RCR was reported to be present, but unconfirmed in the following new counties: Bond, Clinton, Marion, and Montgomery (Robert Bellm, pers. comm.; Fig. 2.3). No samples were received from the request letter for samples in 2021.

**Plant Clinic report of RCR in Illinois.** Christian County had one positive RCR sample in 2018 (Diane Plewa, pers. Comm.) In 2019, the clinic confirmed Pike County samples from

2018 (Kleczewski et al. 2019), but new confirmations were made from plants in 2019. In 2020, the clinic confirmed six positive samples from three counties: Bureau (one), Effingham (one), and Livingston (four). In 2021, the clinic confirmed five positive samples from each of Ford, Kankakee, Tazewell, and Woodford Counties, and one from an unknown location (Fig. 2.3).

## **Discussion**

Since 2018, 14 counties have been identified with RCR in Illinois. It is not clear from the survey results so far that there is any discernable distribution pattern in the state. One might have thought that the distribution pattern be similar to other soilborne soybean diseases that moved from the southern part of the state northward. This includes the distribution of soybean cyst nematode (SCN) that moved from the south to north in the US and in Illinois (Tylka and Maret 2021), and that of sudden death syndrome (Hartman et al. 1995), which also started in the southern states and moved northward. Further surveys in Illinois are needed especially over time to determine the rate of spread of the disease and to determine if there are any patterns associated with that spread. In addition to Illinois, more intense surveys would be useful in those states that already have reported RCR, and in those states that are bordering states with confirmed cases.

Along with the larger picture of state to state or county to county spread of RCR, there is also micro spread within a smaller unit like a field or a few fields. To my knowledge, there doesn't appear to be any studies that have focused on field distribution of this disease. The field patterns of RCR could be similar to the disease pattern of SDS and other soil borne diseases by how the disease is clustered throughout the field (Hartman et al. 1995). The field pattern can provide some clues as to how the pathogen might be moving and include root-to-root spread, or by water or cultivation patterns in the field (Jardine 2020).

I suspect that the distribution of RCR in Illinois is due to fungal inoculum being moved by flooding among fields, contamination of animals, and machinery movement. It is likely that the distribution of RCR in Illinois may be mainly due to movement of farm machinery as was documented in farm equipment moved from Louisiana to Mississippi (Allen 2013). However, there are other possibilities as SCN and other pathogens have been reported to be spread by snow geese (Mason et al. 1993). In any event, the pathogen causing RCR is more prevalent in peanut growing regions of states in the southern United States. It seems possible that *C. ilicicola* may be moving from south to north similar to soybean cyst nematode (Tylka and Marrett 2017) and sudden death syndrome (Roy et al. 1997). Soybean cyst nematode was originally found in North Carolina in 1954 (Winstead et al. 1995) then moved north to as far as Canada in 2017 (Tylka and Marrett 2017). Sudden death syndrome was first observed in Arkansas in 1971 and moved northward and now occurs in all the states in the north-central region of the United States and Canada (Hartman et al. 1995; Tylka and Marrett 2017).

Although *C. ilicicola* has not been reported to be seedborne in soybeans, it is seedborne in peanuts. In peanuts, it is recommended that seed production in infected fields be discontinued and if seed lots are known to be infected with the pathogen, the seeds should not be planted (Shew 2018). The production of resistant cultivars is also recommended in peanuts and this resistance was reported to be more effective than chemical treatment to avoid CBR losses (Anco and Chapin 2017). The commonly grown resistant cultivar Bailey doesn't usually need fumigation or in-furrow fungicides. For susceptible varieties, the use of Propulse and Proline as in-furrow treatment could be used (Anco and Chapin 2017).

## Figures

2021 United States Red Crown Rot Distribution by State

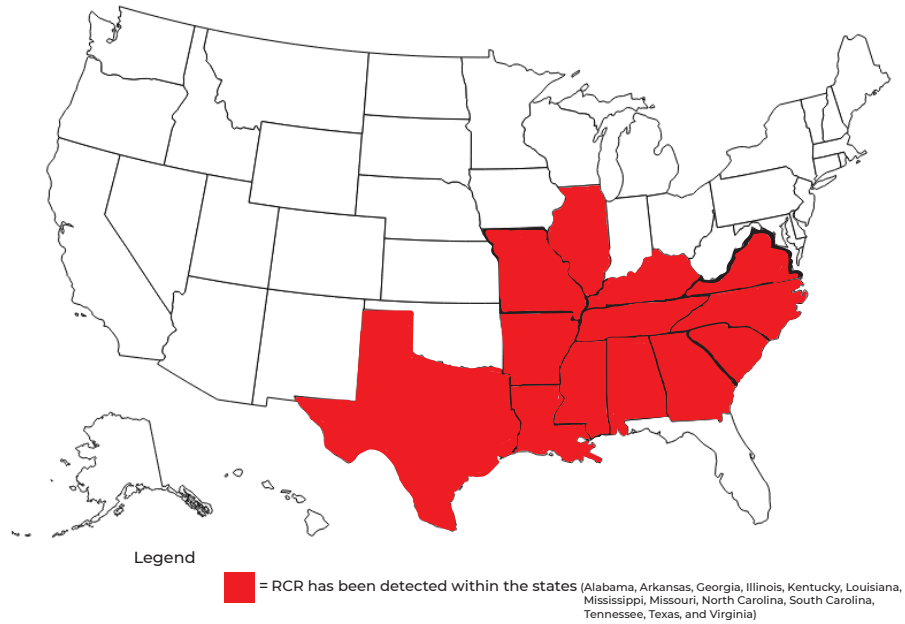


Fig. 2.1. RCR distribution throughout the United States. RCR hasn't been confirmed in TN, but it was found in a research plot (pers. comm. Heather Kelly). In MO, RCR is listed as an emergence disease (Bissonnette et al. 2021), but the distribution is unknown (pers. comm. Kaitlyn Bissonnette; Courtesy of Seth Turner).



Fig. 2.2. Red perithecia on base of soybean plant. (Right picture courtesy of Nathan Kleczewski).

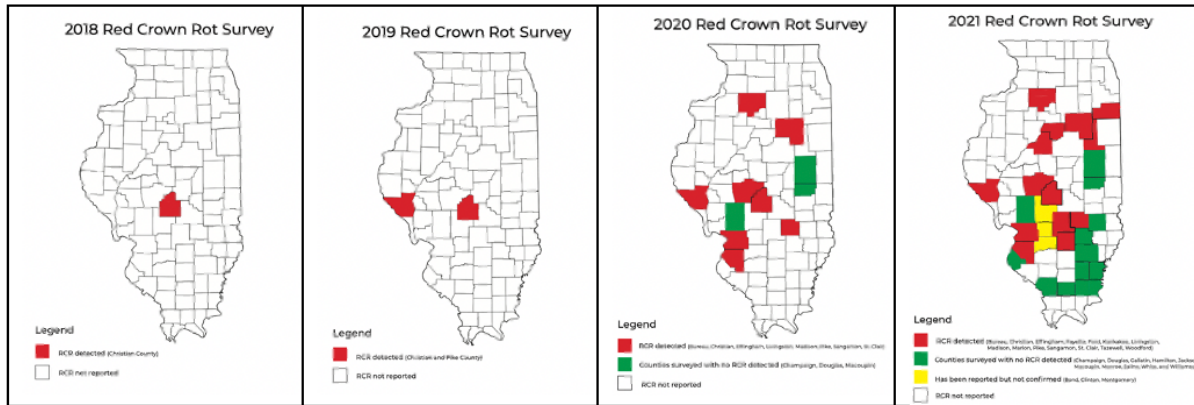


Fig. 2.3. Red crown rot (RCR) distribution maps in Illinois from 2018 through 2021. Counties highlighted in red have been confirmed based on the occurrence of red perithecia on the stems with RCR. The 14 counties confirmed counties include Bureau, Christian, Effingham, Fayette, Ford, Kankakee, Livingston, Madison, Marion, Pike, Tazewell, Sangamon, St. Clair, and Woodford. Counties highlighted with yellow have been reported to have RCR based on (Robert Bellm. Pers. Comm.). The counties highlighted in green were surveyed for RCR, but the disease was not observed. The counties in white were not surveyed (Courtesy of Seth Turner).

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## CHAPTER 3: Soybean fungicide seed treatments control root rot and seedling mortality caused by *Calonectria ilicicola*

### Abstract

*Calonectria ilicicola* is a soilborne fungus known to cause red crown rot (RCR) in soybean and to cause *Cylindrocladium* black rot (CBR) in peanuts. RCR symptoms include pre- and post-emergence damping off, tap root rot, and reddening of the crown area that often becomes encrusted with red perithecia later in the season. The objective of this research was to determine if soybean fungicide seed treatments could reduce the level of pre- and post-emergence damping off and root rot in soybean caused by *C. ilicicola*. Seed treatments and a non-treated control were evaluated by pouring a slurry of the fungus over the seeds at planting. Data on stand counts, root rot ratings, and plant dry weights were recorded. Overall, based on these three variables measured, CruiserMaxx® Vibrance® + Saltro® provided the best treatment results that were equal to the non-inoculated control without any seed treatment in stand counts, root rot ratings, and plant weights. These results indicate that seed treatments provide some control of the pre- and post-emergence damping off including root rot caused by *C. ilicicola* and could be effective for those growers that encounter stand establishment problems due to *C. ilicicola*.

### Introduction

Red crown rot (RCR) of soybean, caused by *Calonectria ilicicola*, was discovered in the US in 1972 (Kim et al. 1998). The foliar symptoms appear similar to the foliar symptoms of sudden death syndrome, brown stem rot, and stem canker (Padgett et al. 2015). Foliar symptoms of RCR begin at flowering and progress through the rest of the season. Infected plants showing foliar symptoms often die prematurely. Infected plants often have red perithecia surrounding the

lower stems, nodules, and roots. In addition, the pathogen infects soybean seedlings causing root rot and pre- and post-emergence damping off. The two main types of fungicide seed coat treatments used are a contact fungicide, which is used as a protectant on the seed surface or a systematic fungicide seed treatment that is absorbed by the seedling to fight or kill the pathogen inside the plant and this type of treatment has a longer residual than contact fungicides (Giesler and Miller 2017).

Soybean growers have limited management options for controlling RCR since there are no cultivars advertised with resistance or fungicide labeled for use. It is not known if fungicides applied to soybean seeds would have any efficacy since there is limited research on the use of fungicide seed treatments to control at least the pre- and post-emergence damping off caused by *C. ilicicola*. A recent study reported that seed treated (a.i. ILEVO<sup>®</sup> or CruiserMaxx<sup>®</sup>+ Saltro<sup>®</sup>) reduced the percent of root rot in seedling tests conducted under controlled conditions (Kleczewski and Geisler 2022; Table 3.1). If this treatment is effective in the field, this would add an additional management strategy for growers and could provide benefits such as increased germination and emergence and may even increase plant vigor and protect the plant and the roots from *C. ilicicola* and other pathogens.

In peanuts, soil and seed fungicide treatments are known to reduce the effects of *C. ilicicola* in susceptible varieties (Anco and Chapin 2016). In soybean, *C. ilicicola* has been considered a soybean seedling disease (Bissonnette et al. 2021), and the use of fungicide seed treatments could suppress the impact of *C. ilicicola* and provide control of pre- and post-emergence damping off. Common soybean seed fungicide treatments, like ILEVO<sup>®</sup> that is labeled to control of SDS and SCN, and Saltro<sup>®</sup> that are labeled for control of SDS have been successfully used to control pre- and post-emergence damping off caused by *C. ilicicola*

(Kleczewski and Geisler 2022). It is not known if these fungicides are effective past the pre- and post-emergence damping off stage since *C. ilicicola*, like the SDS causing pathogen, produces late-season foliar symptoms. My objectives were to determine the efficacy of soybean fungicide seed treatments to protect seedlings from pre- and post-emergence damping off and root rot caused by *C. ilicicola*.

## **Materials and methods**

**Plant materials.** The soybean cultivar S30MAX (Syngenta Crop Protection, Basel, Switzerland) was used to determine if fungicide seed treatments control pre- and post-emergence damping off and root rot caused by *C. ilicicola*. The treated seed was kindly provided by Dave Thomas, Syngenta Crop Protection.

**Inoculum preparation and inoculations.** The inoculum for all experiments was the isolate IL-Pike-2018, which was recovered from the initial report of the disease in Illinois (Kleczewski et al. 2019). A 1 cm diameter plug from a stock culture was transferred to each of 10 plates containing 98 mm potato dextrose agar (PDA; 19.5 g per 500 ml). Plates were wrapped with Parafilm and placed in an incubator without light at 25°C for 14 days. To make a slurry, whole colonies removed from 10 plates were added to one liter of DI water and pulsed in a blender for 10 seconds. To make the non-inoculum control, the same procedure was used as described but with only PDA from 10 plates and water added to the blender.

**Experimental design and fungicide seed treatments.** This experiment was set up as a randomized complete block design with five replicated blocks. Each experimental unit consisted of a 12.7 cm diameter pot. Each pot was filled with 180 g of Berger BM6 medium. For each pot, eight seeds were placed in a circle on top of the medium and either a 15 mL slurry of agar (control) or a fungal slurry was poured gently on top of the seeds. An additional 30 g of BM6

medium was added to cover the seeds. Saucers (15 cm diameter) were placed under each pot and the medium was kept moist by watering the pots above followed by bottom filling with water keeping the saucers wet with 1 cm deep of water. The plants were watered daily if needed. The greenhouse temperature ranged from 23°C to 27°C and from 21°C to 24°C during the day and night, respectively. Supplemental lighting ran from 6:30 am to 6:30 pm, unless the outside light intensity reached 400 w/m<sup>2</sup> then the lights would turn off. The shade curtains would begin to close at 500 w/m<sup>2</sup> then fully close at 800 w/m<sup>2</sup>.

The treatments included: (i) non-treated and non-inoculated soybean seed, (ii) CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> (thiamethoxam, mefenoxam, fludioxonil, and sedaxane) with *C. ilicicola*, (iii) CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> and Saltro<sup>®</sup> (pydifumetofen) with *C. ilicicola*, (iv) CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> and ILEVO<sup>®</sup> (fluopyryram) with *C. ilicicola*, (v) Acceleron<sup>®</sup> (metalaxyl, pyraclostrobin, fluxapyroxad, and imidacloprid) with *C. ilicicola*, (vi) Intego<sup>®</sup> Suite (clothianidin, ethaboxam, ipconazole, and metalaxyl) with *C. ilicicola*, (vii) Obvius<sup>®</sup> Plus<sup>®</sup> and (fluxapyroxad, pyraclostrobin, metalaxyl, thiophanate-methyl, and imidacloprid) with *C. ilicicola*, (viii) non-treated soybean seed inoculated with *C. ilicicola*, and (ix) CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> without *C. ilicicola* (Tables 3.1 and 3.2).

Seeding emergence was counted at 21 days after planting. On day 25, roots from each pot were washed and rated for necrosis. The visual root rot rating per pot was based on a continuous scale of 0-100%. Plants with scores of “0” showed no signs of necrosis whereas those scored “100” had all roots rotted. After the ratings, whole plants from each pot were placed into a dryer at 50°C for one week then weighed. This experiment was completed twice.

**Statistical analyses.** Prior to analyses all data were assessed for normality (R Studio). The data were subjected to an analysis of variance using a mixed model with experiments as a

random factor and treatments as the fixed factor using R studio. Least-square mean separations were conducted by F-tests with a Levene test followed to evaluate the relationship among treatments at  $\alpha = 0.05$ .

## Results

Based on the analysis of variance, there was no interaction between experiments and the treatments so the data for the two trials were combined for analysis of variance. The treatments had a significant effect on stand count ( $P < 0.019$ ), root rot ratings ( $P < 0.035$ ), and dry weights ( $P < 0.009$ ) (Table 3.3).

At 21 days after sowing, Treatment 4 (CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> + ILEVO<sup>®</sup> with inoculum) had the highest stand count (98.2%) which was not significantly different from other treatments except for Treatment 5 (Acceleron<sup>®</sup>, inoculated) and Treatment 8 (no fungicide seed treatment, inoculated) (Table 3.4). Treatment 8 (no fungicide, inoculated) also had the lowest stand count (24%) and was significantly lower than all the other treatments (Table 3.4).

Treatment 8 (no fungicide, inoculated) had the highest root rot rating (89%) and both Treatment 1 (no fungicide seed treatment, non-inoculated) and Treatment 9 (CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup>, non-inoculated) had the lowest root rot (1%) (Table 3.5). Treatment 3 (CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> + Saltro<sup>®</sup>, inoculated) had a root rating of 19%, which was not significantly different from the non-inoculated treatments. Some treatments like Treatments 6 (Intego<sup>®</sup> Suite, inoculated), 5 (Acceleron<sup>®</sup>, inoculated), and 7 (Obvius<sup>®</sup> Plus, inoculated) had high root rot ratings that did not differ from Treatment 8 (no fungicide, inoculated).

Treatment 8 (no fungicide, inoculated) had the lowest plant dry weight (0.02 g) and Treatment 1 (no fungicide, non-inoculated) had the highest weight (2.14 g) (Table 3.5). Treatments 9 (CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup>, non-inoculated), 3 (CruiserMaxx<sup>®</sup> Vibrance<sup>®</sup> + Saltro<sup>®</sup>,

inoculated) and 4 (CruiserMaxx® Vibrance® + ILEVO®, inoculated) that had weights of 2.00 g, 1.96 g, and 1.4 g, respectively, were not significantly different from Treatment 1 (no fungicide, non-inoculated). Other treatments like Treatments 6 (Intego® Suite, inoculated), 5 (Acceleron®, inoculated), 7 (Obvius® Plus, inoculated) and 2 (CruiserMaxx® Vibrance®, inoculated) had weights that did not differ significantly from Treatment 8 (no fungicide, inoculated).

## Discussion

To date there is no labeled fungicides for use on soybean for control of RCR. One approach to control pre- and post-emergence damping off caused by RCR is to use seed treatment. In my experiment, stand counts taken at 21 days after sowing were not significantly different from the non-inoculated control for all the treatments except for Acceleron® and the inoculated, non-fungicide treatment. For root ratings, only one treatment, CruiserMaxx® Vibrance® + Salto®, had root ratings that were not different from the non-fungicide, non-inoculated treatment. For plant dry weights, the treatments with CruiserMaxx® Vibrance Beans (non-inoculated), CruiserMaxx® Vibrance® + Salto®, CruiserMaxx® Vibrance® + ILEVO® had weights that were not significantly different from the non-treated + non-inoculated treatment. Overall, based on these three variables measured, CruiserMaxx® Vibrance® + Salto® was the best treatment since it provided protection equal to the non-inoculated control in stand counts, root rot ratings, and plant weights.

A recently published study showed ILEVO® + Salto® to be effective in controlling pre- and post-emergence damping off of soybean caused by *C. ilicicola* (Kleczewski and Giesler 2022). In my study, CruiserMaxx® Vibrance® + Salto® and CruiserMaxx® Vibrance® and ILEVO® were also effective treatments in both stand count and root rot. In my study, CruiserMaxx® and Acceleron® was not effective with controlling root rot of soybean, but used

with other products, such as ILEVO® and Saltro®, the results were similar to non-inoculated controls. As expected, the fungicide seed treatments with insecticides (Intego® Suite, Acceleron®, and Obvius® Plus) did not add any additional control based on stand counts or root rot ratings.

In other crops like peanuts, *C. ilicicola* control has been effective by using fungicides and, in the past, fumigation practices (Anco and Chapin 2016). When planting susceptible peanut cultivars, recommendations include using in furrow fungicides containing prothioconazole (Proline®; Bayer crop science) and fluopyram and prothioconazole (Propulse® BASF) (Anco 2017). Although the current labels of fungicide seed treatments for soybean do not yet include *C. ilicicola*, it is likely that with more field efficacy studies completed in the next several years, labeling will include red crown rot.

## Tables

**Table 3.1.** Trade name, chemical name, company website, and target of use for soybean seed treatments using this study.

Trade name	Chemical name	Company website	Target
CruiserMaxx <sup>®</sup> Vibrance Beans <sup>®</sup>	thiamethoxam, mefenoxam, fludioxonil, and sedaxane	Syngenta: <a href="https://www.syngenta-us.com/seed-treatment/cruisermaxx-vibrance-beans">https://www.syngenta-us.com/seed-treatment/cruisermaxx-vibrance-beans</a>	Use to protect against damage from early-season insects, soilborne and seedborne diseases including those caused by species of <i>Pythium</i> , <i>Fusarium</i> and <i>Rhizoctonia</i>
Saltro <sup>®</sup>	pydiflumetofen	Syngenta: <a href="https://www.syngentaus.com/seedtreatment/saltro?gclid=Cj0KCCQjwuMuRBhCJARIsAHXdngNI6kyfJPzWQeccVW83u0JwKkTXsbpXSzENuVKEisALH8K0UOJFqmYaAvwqEALw_wcB">https://www.syngentaus.com/seedtreatment/saltro?gclid=Cj0KCCQjwuMuRBhCJARIsAHXdngNI6kyfJPzWQeccVW83u0JwKkTXsbpXSzENuVKEisALH8K0UOJFqmYaAvwqEALw_wcB</a>	Provides sudden death syndrome (SDS) protection
ILEVO <sup>®</sup>	flupyram	BASF: <a href="https://agriculture.basf.us/cropprotection/products/seedtreatment/ilevo.html?at_medium=customPaidSearch&amp;at_campaign=Seed_Brand_ILEVO&amp;at_custom1=Google&amp;at_custom2=ILEVO_PPC&amp;at_custom3=&amp;gclid=CjwKCAjwlcaRBhBYEiwAK341jUBgODxQyyU0iPT2NaEhn3OENIDnoYxBkASufWfrAcvA5OsSPCGOfxoCCXwQAvD_BwE&amp;gclid=aw.ds">https://agriculture.basf.us/cropprotection/products/seedtreatment/ilevo.html?at_medium=customPaidSearch&amp;at_campaign=Seed_Brand_ILEVO&amp;at_custom1=Google&amp;at_custom2=ILEVO_PPC&amp;at_custom3=&amp;gclid=CjwKCAjwlcaRBhBYEiwAK341jUBgODxQyyU0iPT2NaEhn3OENIDnoYxBkASufWfrAcvA5OsSPCGOfxoCCXwQAvD_BwE&amp;gclid=aw.ds</a>	Use to protect against SDS and nematodes (SCN)
Acceleron <sup>®</sup>	fungicides and insecticide; metalaxyl, pyraclostrobin, fluxapyroxad, and imidacloprid	Bayer: <a href="https://www.cropsscience.bayer.us/seedgrowth/acceleron/soybean">https://www.cropsscience.bayer.us/seedgrowth/acceleron/soybean</a>	Protects against species of <i>Pythium</i> , <i>Phytophthora</i> , <i>Fusarium</i> , and <i>Rhizoctonia solani</i> along with pests such as bean leaf beetles, early season soybean aphids and seed corn maggots
Intego <sup>®</sup> Suite	clothianidin, ethaboxam, ipconazole, and metalaxyl	Valent: <a href="https://www.valent.com/Products/c4754cf4-024b-4b90-85cc-dbbaab79be48/intego-suite-soybeans">https://www.valent.com/Products/c4754cf4-024b-4b90-85cc-dbbaab79be48/intego-suite-soybeans</a>	Protects against aphids, bean leaf beetle, and species of <i>Pythium</i> , <i>Phytophthora</i> , <i>Rhizoctonia</i> , seed corn maggot
Obvius <sup>®</sup> Plus	fluxapyroxad, pyraclostrobin, metalaxyl, and thiophanate-methye	BASF: <a href="https://agriculture.basf.us/crop-protection/products/seed-treatment/obvius-plus.html">https://agriculture.basf.us/crop-protection/products/seed-treatment/obvius-plus.html</a>	Protects against species of <i>Phytophthora</i> , <i>Pythium</i> , <i>Fusarium</i> , and <i>Rhizoctonia</i>

**Table 3.2.** Treatments listed as their chemical names, amount of fungicide applied per seed, and whether if the treatments were inoculated with *Calonectria ilicicola* or not.

<b>Treatment</b>	<b>mg ai/seed<sup>1</sup></b>	<b>Inoculated<sup>2</sup></b>
Not treated seed	NA	No
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup>	0.0945	Yes
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup> + Saltro <sup>®</sup>	0.075; 0.0945	Yes
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup> + ILEVO <sup>®</sup>	0.15; 0.0945	Yes
Acceleron <sup>®</sup>	0.0118; 0.0118; 0.0077; 0.1181	Yes
Intego <sup>®</sup> Suite	0.1002	Yes
Obvius <sup>®</sup> Plus	0.0535; 0.0947	Yes
Not treated seed	NA	Yes
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup>	0.0945	No

<sup>1</sup> See Table 3.1 for chemical names associated with these products.

<sup>2</sup> Inoculated with the *C. ilicicola* during the time of each experiment.

**Table 3.3.** Analysis of variance for percentage stand at 21 days after planting, root rot ratings, and whole plant dry weight 32 days after planting for plants that received fungicide and *Calonectria ilicicola* inoculation treatments

<b>Source</b>	<b>Df</b>	<b>F ratio for stand count at 21 days<sup>1</sup></b>	<b>F ratio for root rot ratings<sup>1</sup></b>	<b>F ratio for whole plant dry weight<sup>1</sup></b>
Experiment	1	2.2*	0.1	2.2
Treatment	8	23.4***	11.2**	6.9**
Experiment x treatment	8	2.3	0.1	0.6

<sup>1</sup>Asterisks: \*, \*\*, and \*\*\* indicate significant differences at  $P < 0.05$ , 0.01, and 0.001, respectively.

**Table 3.4.** Percentage stand at 21 days after sowing soybean seeds treated or not treated (treatments #1 and 8) with a seed treatment and inoculated with *Calonectria ilicicola* or not inoculated (treatments #1 and #9).

<b>Treatment</b>	<b>Stand count (%)</b>
CruiserMaxx <sup>®</sup> Vibrance Beans <sup>®</sup> + ILEVO <sup>®</sup>	98.2 d
Non treated seed + non inoculated	94.5 cd
CruiserMaxx <sup>®</sup> Vibrance Beans <sup>®</sup> + Saltro <sup>®</sup>	94.5 cd
CruiserMaxx <sup>®</sup> Vibrance Beans <sup>®</sup> + non inoculated	92.7 b-d
CruiserMaxx <sup>®</sup> Vibrance Beans <sup>®</sup> +inoculated	89.1 b-d
Obvius <sup>®</sup> Plus	83.6 b-d
Intego <sup>®</sup> Suite	72.7 bc
Acceleron <sup>®</sup>	70.9 b
Non treated seed + inoculated	23.6 a

<sup>1</sup>Stand count based on eight plants per pot per five replications.

**Table 3.5.** Soybean seeds treated or not treated (treatments #1 and 8) with a seed treatment and inoculated with *Calonectria ilicicola* at sowing except for treatments #1 and #9. Means of root rot rating and whole plant weight 25 days after inoculation.

<b>Treatment</b>	<b>Root rot (%)<sup>1</sup></b>	<b>Whole plant dry weight (g)</b>
Non-treated + inoculated	88.6 d	0.02 a
Intego <sup>®</sup> Suite	82.7 d	0.55 ab
Acceleron <sup>®</sup>	75.0 cd	0.69 ab
Obvius <sup>®</sup> Plus	78.6 d	0.82 a-c
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup> +inoculated	54.5 c	0.92 a-d
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup> + ILEVO <sup>®</sup>	25.0 b	1.40 b-e
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup> + Saltro <sup>®</sup>	18.6 ab	1.96 c-e
CruiserMaxx <sup>®</sup> Vibrance <sup>®</sup> + non-inoculated	0.9 a	2.00 de
Non-treated + non-inoculated	0.9 a	2.14 e

<sup>1</sup>Plants with scores of “0” showed no signs of necrosis whereas those scored “100” had all roots rotted based on 8 plants per pot per 5 replications.

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## CHAPTER 4: Evaluation of soybean germplasm for resistance to *Calonectria ilicicola*

### Abstract

Red crown rot (RCR) of soybean is caused by *Calonectria ilicicola*, a soilborne fungus. Red crown rot has been reported in the United States, Asia, and Australia. There has been a dearth of information about sources of resistance to this disease or about resistant cultivars for growers use. The goal of this research was to discover sources of resistance in soybean to *C. ilicicola*. My objectives were to evaluate current breeding lines/cultivars from the University of Illinois soybean breeding program and a subset of ancestral soybean lines. Three experiments were completed, one for the breeding lines/cultivars and two for the ancestral lines, in a greenhouse under inoculated, controlled conditions. For the breeding lines/cultivar experiment, LD10-10219 had the lowest root rot rating. In the first ancestral lines evaluation which included older modern cultivars along with ancestors, Corsoy 79 and Williams 82 generally had the lowest root rot ratings. In the second ancestral lines evaluation, Mukden, Tokyo, PI 54610, and Dunfield had the lowest root rot ratings. The significant differences among genotypes observed in these evaluations show that some genotypes carry genes for resistance to *C. ilicicola*.

### Introduction

Soybean is an economically important crop in the United States and globally as it provides protein, oil, and income through domestic use and exports. In the United States, the cash value of soybean exports was \$US 46.1 billion with China, Mexico, Egypt, Netherlands, and Japan as the five top importers (NASS 2022). In 2020, Illinois was the number one state for soybean production with 16.4 million metric tons (604 million bushels; NASS 2022).

Red crown rot (RCR) of soybean is caused by *Calonectria ilicicola*, a soilborne fungus. The pathogen was originally found on peanut in Georgia in 1965 (Gia et al. 2017) and first

reported on soybean in the United States in 1972 (Kim et al. 1998). RCR has been reported in southern states in the United States (Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas, and Virginia), as well as in Australia and parts of Asia (Padgett et al. 2015) and more recently in Illinois (Kleczewski et al. 2019) and Kentucky (Blake 2021). In soybean, *C. ilicicola* causes pre- and post-emergence damping off as well as root rot and interveinal foliar chlorosis and necrosis after flowering. Infected plants die prematurely reducing seed production and seed weight (Akamatsu et al. 2020). Management of RCR relies on culture practices that include rotation with rice or other grain crops, fallow periods to decrease inoculum, avoiding planting during wet periods, and tillage (Akamatsu et al. 2020).

Host plant resistance has been used to manage many soybean diseases. Most if not all soybean commercial cultivars in the United States have resistance to one or more diseases, usually Phytophthora root and stem rot and/or soybean cyst nematode (Hartman 2015). There is a long history of using host resistance as part of a management strategy to control diseases. Some public cultivars, like Corsoy 79 and Williams 82, were bred to be resistant to certain races of the pathogen that causes Phytophthora root and stem rot. Some of the resistance used today was found in a set of ancestors of commercial public cultivars (Gizlice et al. 1994). This soybean ancestral set or subsets have been used to screen for a number of different diseases including anthracnose (Yang and Hartman 2017), charcoal rot (Pawlowski et al. 2015), soybean mosaic virus (Wang et al. 2005), and Rhizoctonia root and hypocotyl rot (Bradley et al. 2001).

Currently, there is a lack of information on host plant resistance against RCR in soybean. There are only two previous studies on soybean resistance to *C. ilicicola*. In one study, cultivars Asgrow 7986, Braxton, Cajun, and Forrest were ranked least susceptible to *C. ilicicola* in the greenhouse based on necrotic stem lesions, leaf symptoms, and plant wilt (Kim et al. 1998).

These resistant cultivars were then tested in the field, and none were deemed resistant due to having one or more of the following symptoms or signs: perithecia on stems, leaf symptoms, or reddish coloration on stem (Kim et al. 1998). In the second study, cultivars Enrei, Fukuyuyaka, Jack, and Peking were all rated as susceptible with high root rot scores (Jiang et al. 2020). The objective of my study was to find sources of resistance to *C. ilicicola* in soybean. I evaluated 25 cultivars/elite breeding lines and 27 entries selected mostly from the soybean ancestral set that represent ancestors of modern-day public cultivars (Gizlice et al. 1994).

### **Materials and methods**

**Inoculum.** The *C. ilicicola* inoculum used in the experiments was from the isolate IL-Pike-18. The isolate was originally collected from soybean plants from Pike County, Illinois (Kleczewski et al. 2019). Each petri plate represented a colony of *C. ilicicola*, which was grown on full strength PDA for 14 days and wrapped with parafilm. At 14 days, the colonies grew to cover the full diameter of the plates. Before each experiment, each colony was analyzed for any contamination, unusual growth patterns or colors, and broken parafilm seals.

**Evaluation of cultivars/elite breeding lines.** Seeds of 25 breeding lines/cultivars from the University of Illinois Soybean Breeding Program were obtained from Brian Diers (University of Illinois; Table 4.1). The 25 genotypes were selected because they were released as commercial cultivars or were being considered for release (Brian Diers, per. comm.). For each entry, five seeds were planted in each 12.7 cm diameter plastic pot that had a 11.45 cm diameter coffee filter placed on the bottom. Each pot was filled with a total of 235 g of dry coarse torpedo sand with 5.5 g 13-13-13 Osmocote fertilizer mixed into the sand. Next, 50 g of sand was added to the pot. A two-week-old colony of *C. ilicicola* (IL-Pike-18 isolate) grown on PDA was removed from the 9.8 cm diameter plate and placed on top of this layer which was then covered with 75 g

of sand before placing five seeds in the pot. The seeds were covered with 130 g of sand and watered daily. Stand counts were completed 7 days after planting and converted to percent stand based on seeding rate of five plants per pot. The plants were removed from the pots at 14 days after planting to record root rot. The visual root rot rating was based on a continuous scale of 0-100% where a 0 represents no root rot symptoms to 100 where all the roots were rotted.

The greenhouse climate settings were 24°C – 26.7°C during the day and 22°C – 24°C at night. The additional lights (LED white-full spectrum lamps) were on for 12 hours daily and would turn off automatically if the outside light intensity reached 400 w/m<sup>2</sup>. The shades would start to close at 500 w/m<sup>2</sup> and fully close at 800 w/m<sup>2</sup>.

The experiments were implemented with a completely randomized design with three replications and were repeated with a different randomization of the entries.

**Evaluation of a soybean ancestral set.** Twenty-seven entries selected mostly from the soybean ancestral set were evaluated (Table 4.2). Seeds were obtained from Dr. Adam Mahan, the curator of the national soybean germplasm collection at Urbana, IL. The experimental set up and design were identical to the previously described experiment. This set was separated into two experiments due to lack of space during the experimental run. Experiment one had 14 PIs with the two checks and the second experiments had the 13 remaining PIs. These experiments were also repeated twice. Data on stand count and root rot were collected and analyzed as previously reported for the elite breeding lines.

**Data analysis.** Data were tested for normality using the distribution function and an arcsine square root transformation was used to normalize percentage data. Data were analyzed using an analysis of variance (JMP v16). If F-tests were significant ( $P < 0.05$ ), means were

compared by student t-test to compare each pair ( $\alpha = 0.05$ ). A pair-wise correlation comparison was used to assess the relationship of data over repeated experiments.

## Results

**Evaluation of cultivars/elite breeding lines.** The data for the two trials were not combined based on a significant trial x entry effect and the lack of correlation between the entries of the two trials. When analyzed individually, there was a significant effect of entry for root rot rating but not for percent stand count after 7 days. Root rot ratings ranged from 26.7 to 96.7% and 11.7 and 90.0% for trials one and two, respectively (Table 4.3). For trial 1, the lowest rating was for LD12-459 (26.7%) and did not differ significantly from 11 other entries. For trial 2, the lowest rating was for LD15-467 (11.7%) and this did not differ significantly from seven other entries. Only one entry, LD10-10219, was consistently among the entries with the lowest root rot rating in both trials. On the other hand, there were three entries, LD15-5789800, LD07-3395bf, and LD12-10534, that were among the entries with the highest root rot rating in both trials. Based on arithmetic means across the trials, the five lowest entries were LD10-10219 (37%), LD15-5782791 (43%), LD15-467 (43%), LD18-15909R1a (48%), and LD12-459 (48%).

**Evaluation of a soybean ancestral set.** These two experiments were analyzed separately and two trials within each experiment were not combined based on a significant trial x entry effect and the lack of correlation between data from the two trials within each experiment.

In the first experiment, the root rot ratings ranged from 31.7 % to 88.3% and 2.7% to 81.6% for trials one and two (Table 4.4). In trial one, Ogden had the lowest root rot rating (32%), which was not significantly different to six other entries. In trial two, Corsoy 79 had the lowest root rot rating (3%), which was not significantly different to Williams 82 (23%). In both of the

trials, Williams 82 and Corsoy79 were in the group with the lowest root rot ratings and had arithmetic means of 31 and 33%, respectively.

In the second experiment, the root rot ratings ranged from 5.0% to 88.9% and 9.0% to 79.3% for trials one and two, respectively (Table 4.5). In trial one, Tokyo had the lowest root rot rating (5%), which was not significantly different from five other ancestral lines. In trial two, Richland, had the lowest root rot rating (9%) which was not significantly different from six other ancestral lines. Based on arithmetic means, the entries with the lowest root rot rating were Mukden (11%), Tokyo (15%), and PI54610 (17%).

## **Discussion**

Finding sources of resistance in soybean to *C. ilicicola* is important since there are currently no reported sources of resistance. I tested elite breeding lines that are commercialized or are being considered for commercialization and a subset of mostly ancestral lines for resistance to RCR. In these experiments, I found good separation of entries based on root rot ratings. I did have some trouble with consistency when I repeated the experiments, but overall, there were a few entries in each experiment that had low root rot ratings. There are still many unanswered questions, including about methodology on how to obtain better consistency from run to run within an experiment, and maybe even more importantly, do results from the method I used correlate with resistance in the field. Germplasm identified as resistant in my assays need to be further tested and field verified.

In the set of breeding lines tested, LD10-10219 had the lowest root ratings. After reviewing the traits related to this line and the other four lines (LD15-5782791, LD15-467, LD18-15909R1a, and LD12-459) with lower RCR root ratings, there did not appear to be any trend related to pod color, maturity group, traits, or parentage. All of these lines have genes for

SCN resistance and four of them have Phytophthora root and stem rot resistance. If these entries do in fact have RCR resistance based on further testing, these lines may be recommended over other cultivars or elite breeding lines for environments where RCR may be problematic.

In the first ancestral set, the two older modern cultivars (Williams 82 and Corsoy79) had the lowest root rot ratings. Both of these have Phytophthora resistance (Dorrance et al. 2004). In the second set, Mukden, Tokyo, PI54610 and Dunfield had the lowest root rot rating. Cumulatively, these represent about 15% of parentage to modern public cultivars (Gizlice et al. 1994). In contrast, the ancestors Lincoln (first progeny) and Mandarin (Ottawa) had high root rot ratings, and they cumulatively represent about 30% of parentage to modern public cultivars (Gizlice et al. 1994). Although it is not proven, it is likely that the genetic background of modern-day soybean public cultivars leans toward susceptibility to *C. ilicicola* as opposed to being more resistant to this pathogen. In addition, since there has been no breeding for resistance and no known selection away from susceptibility to *C. ilicicola*, it is likely that most soybean cultivars grown by farmers today are susceptible to *C. ilicicola*.

To manage RCR of soybean, it will likely take multiple approaches including crop rotation, seed treatments, and resistance working together to prevent major losses in production. In terms of resistance, peanut breeders have been successful in finding sources of resistance to *C. ilicicola* (Hollowell et al. 2008), and it has been stated that the use of resistant peanut varieties are more effective than fungicide treatment (Anco and Chapin 2016). I tested a total of 52 soybean entries and what is needed now is to confirm the screening methodology, test more soybean entries, confirm the resistance, and develop a breeding strategy to incorporate the resistance into elite breeding lines and to cultivars that farmers will grow.

## Tables

**Table 4.1.** Evaluation of resistance for soybean breeding lines and cultivars inoculated with *Calonectria ilicicola*, the cause of red crown rot.

Entries <sup>1</sup>	Name	FPhil m <sup>2</sup>	Pod	RM <sup>3</sup>	Traits <sup>4</sup>	Parentage
LD17-6036a	NEW Illini	WGbf	brown	2.6	SCN, Rag1 + 2	U11-614119 x LD14-8003
LD17-3855	NEW Illini	PGy	brown	2.6	SCN	LD10-10198 x LG11-6210
LD17-1887	NEW Illini	MLtbl	brown	2.9	SCN	U11-614119 x LD10-10198
LD18-1855	NEW Illini	PGy	brown	3.0	SCN, <i>Rps</i>	LD11-7311 x LD10-10198
LD17-2946a	NEW Illini	WLtbl	brown	3.1	SCN, Rag1	U11-614119 x LD09-30224
LD18-1867*	NEW Illini	WGy	brown	3.2	SCN, <i>Rps</i>	LD11-7311 x LD10-10198
LD17-8668	NEW Illini	PGy	Tan	3.3	SCN	E11128T x U09-105007
LD18-15909R1a	NEW Illini	PLtbl	brown	3.5	RR1, SCN, <i>Rps</i> , no Rag	LD12-3903 x LD12-15156 R1a
LD18-15377R1a	NEW Illini	PLtbr	brown	3.7	RR1, SCN, <i>Rps</i> , no Rag	LD11-2170 x LD12-15156 R1a
LD18-15869R1a	NEW Illini	PTbl	brown	3.7	RR1, SCN, <i>Rps</i> , no Rag	LD12-3903 x LD12-15156 R1a
LD10-10198	Illini 2643N	PGg	brown	2.6	SCN	LD05-3230 x LD00-3309
LD12-459	Illini 2904N	PGibl	tan	2.9	SCN	LD02- 4485 x LD06-7620
LD11-2170	Illini 3025N	PLtbr	brown	3.0	SCN, SDS, <i>Rps1k</i>	Syngenta 03JR313108 x LD05-3171
LD15-456	Illini 3156N	WLtbl	brown	3.1	SCN, <i>Rps1k</i>	HM09-W084 x LD10-10226
LD10-10219	Illini 3264N	PLtbl	brown	3.2	SCN	LD05-3230 x LD00-3309
LD15-467	Illini 3267N	WLtbl	brown	3.2	SCN, <i>Rps1k</i>	HM09-W084 x LD10-10226
LD15-6762	Illini 3317N	PGbf	tan	3.3	SCN, <i>Rps1c</i>	WN0902577 x SD08CV-2102
LD15-5782791	Illini 3546N	PTbl	brown	3.5	SCN, <i>Rps1c</i>	LD06-7620 x Syngenta 05BR006009
LD15-5776793	Illini 3648N	PLtbl	brown	3.6	SCN, <i>Rps1c</i>	LD06-7620 x Syngenta 05BR006009
LD07-3395bf	Illini 3849N	WGbf	tan	3.8	SCN	WW115926 x LD00-2817
LD15-5789800	Illini 3855N	PLtbl	brown	3.8	SCN, <i>Rps1c</i>	LD06-7620 x Syngenta 05BR006009
LD12-10534	Illini 3989N	WLtbl	brown	3.9	LR SCN	LG04-6000 x (LD00-3309(5) x LD07-5065)
LD15-3818	Illini 4218N	PLtbl	tan	4.2	SCN, <i>Rps1a</i>	LD09-3913 x BN09002129
LD16-2955	Illini 4300N	PLtbl	brown	4.3	SCN	LD07-3395bf x LD10-10219

<sup>1</sup>Entries were provided by University of Illinois' soybean breeding program overseen by Dr. Brian Diers.

<sup>2</sup>Flower color: W = white flower, P = purple flower, M = mixed; P = pubescence color: G = gray, Lt = light tawny; T = tawny; hilum = BF = buff, Y = yellow, BL = black; BR = brown.

<sup>3</sup>RM= relative maturity

<sup>4</sup>Traits: SCN = soybean cyst nematode, SDS = sudden death syndrome, Rag = Resistant Soybean-Aphis glycine, *Rps* = resistance *Phytophthora sojae*, RR1= Roundup Ready 1

**Table 4.2.** Evaluation of resistance for soybean ancestors of modern-day public cultivars inoculated with *Calonectria ilicicola*, the cause of red crown rot.

Name <sup>1</sup>	PI number	MG
Williams 82	PI 518671	III
Corsoy 79	PI 518669	II
Harosoy	PI 548573	II
Ogden	PI 548477	VI
Adams	PI 548502	III
Blackhawk	PI 548516	I
Capital	PI 548311	0
Clark	PI 548533	IV
Hawkeye	PI 548577	II
Perry	PI 548603	IV
Pagoda	PI 548398	00
Scott	PI 548613	IV
Wayne	PI 548628	III
Lincoln	PI 548362	III
Mandarin (Ottawa)	PI 548379	0
CNS	PI 548445	VII
Richland	PI 548406	II
S-100	PI 548488	V
AK (Harrow)	PI 548298	III
Tokyo	PI 548493	VII
PI 54610	PI 54610	VI
Dunfield	PI 548318	III
Mukden	PI 548391	II
Illini	PI 157434	IV
Manitoba Brown	PI 548382	00

<sup>1</sup>Williams 82 and Corsoy 79 are not a part of the modern-day public cultivars ancestral lines Gizlice et al. 1994). Williams (7) x Kingwa = Williams 82. Corsoy (6) x Lee 68 = Corsoy 79. Source: Germplasm Resource Information Network (GRIN) at <https://npgsweb.ars-grin.gov/gringlobal>

**Table 4.3.** Root rot ratings of soybean breeding lines 14 days after inoculation with *Calonectria ilicicola*, the cause of red crown rot.

Trial 1		Trial 2	
Entries <sup>1</sup>	Root rot (%)	Entries <sup>1</sup>	Root rot (%)
LD12-10534	96.7 A	LD15-5789800	90.0 A
LD15-5789800	93.3 AB	LD07-3395bf	86.7 AB
LD16-2955	88.3 A-C	LD15-5776793	85.0 A-C
LD07-3395bf	85.0 A-D	LD17-3855	85.0 A-C
LD18-15869R1a	81.7 A-E	LD17-1887	80.0 A-D
LD17-2946a	80.0 A-E	LD10-10198	76.7 A-E
LD17-8668	80.0 A-F	LD12-10534	75.0 A-E
LD18-15377R1a	80.0 A-F	LD18-1867*	73.3 A-E
LD15-6762	76.7 B-F	LD12-459	70.0 B-E
LD15-3818	75.0 B-F	LD17-6036a	70.0 B-F
LD15-467	73.3 C-G	LD15-456	66.7 B-F
LD17-6036a	71.7 C-G	LD18-15909R1a	66.7 C-F
LD18-1855	70.0 C-G	LD17-2967a	65.0 C-F
LD17-2967a	63.3 C-H	LD18-15377R1a	65.0 C-F
LD10-10198	63.3 D-H	LD11-2170	60.0 D-G
LD15-5776793	50.3 E-H	LD17-8668	60.0 D-G
LD17-3855	50.0 E-H	LD17-2946a	53.3 E-G
LD10-10219	50.0 E-H	LD15-5782791	45.0 F-H
LD15-456	50.0 E-H	LD18-1855	36.7 G-I
LD11-2170	50.0 E-H	LD15-3818	35.0 G-I
LD15-5782791	40.0 F-H	LD15-6762	25.0 H-I
LD18-1867*	36.7 G-H	LD10-10219	23.3 H-I
LD17-1887	30.0 H	LD18-15869R1a	21.7 H-I
LD18-15909R1a	30.0 H	LD16-2955	20.3 H-I
LD12-459	26.7 H	LD15-467	11.7 I

<sup>1</sup>Entries were provided by University of Illinois' soybean breeding program overseen by Dr. Brian Diers.

**Table 4.4.** Root rot ratings of selected ancestors of modern-day public soybean cultivars 14 days after inoculation with *Calonectria ilicicola*, the cause of red crown rot (first set).

Trial 1			Trial 2		
Entries <sup>1</sup>	Root rot (%)		Entries <sup>1</sup>	Root rot (%)	
Adams	88.3	A	Harosoy	81.6	A
Clark	83.3	A-B	Wayne	75.0	A-B
Harosoy	80.0	A-B	Perry	73.3	A-B
Hawkeye	78.3	A-C	Scott	71.7	A-B
Mandarin (Ottawa)	78.3	A-C	Mandarin (Ottawa)	71.7	A-B
Lincoln	71.7	A-D	Lincoln	70.0	A-B
Capital	70.0	B-E	Pagoda	70.0	A-B
Perry	68.3	B-E	Adams	66.7	A-B
Blackhawk	58.3	B-F	Blackhawk	65.0	A-B
Corsoy 79	58.3	B-F	Ogden	60.0	A-B
Pagoda	50.0	C-F	Clark	60.0	A-B
Wayne	41.7	D-F	Hawkeye	60.0	A-B
Williams 82	41.7	D-F	Capital	53.3	B-C
Scott	36.7	E-F	Williams 82	23.3	C-D
Ogden	31.7	F	Corsoy 79	2.7	D

<sup>1</sup>Seeds were obtained from Dr. Adam Mahan, the curator of the Soybean Germplasm Collection, Urbana, Illinois.

**Table 4.5.** Root rot ratings of selected ancestors of modern-day public soybean cultivars 14 days after inoculation with *Calonectria ilicicola*, the cause of red crown rot (second set).

Trial 1			Trial 2		
Entries <sup>1</sup>	Root rot (%)		Entries <sup>1</sup>	Root rot (%)	
AK (Harrow)	88.9	A	AK (Harrow)	79.3	A
S-100	51.3	B	Illini	72.7	A
Richland	44.7	B-C	CNS	62.0	A-B
Manitoba Brown	31.3	B-D	Dunfield	31.3	B-C
CNS	27.0	C-E	PI 54610	24.7	C
Dunfield	24.7	C-E	Tokyo	24.7	C
Illini	20.3	D-E	S-100	20.3	C
Mukden	9.3	D-E	Manitoba Brown	16.0	C
PI 54610	9.3	D-E	Mukden	13.7	C
Tokyo	5.0	E	Richland	9.0	C

<sup>1</sup>Seeds were obtained from Dr. Adam Mahan, the curator of the Soybean Germplasm Collection, Urbana, Illinois

**Table 4.6.** Soybean entries in a subset of ancestors of modern-day public cultivars plus Williams and Corsoy that showed the least amount of root rot when inoculated with *Calonectria ilicicola*, the cause of red crown rot, and their associated disease resistance traits.

Name	Plant introduction number <sup>1</sup>	Reported resistance <sup>2</sup>
Williams 82	518671	<i>Rps</i>
Corsoy 79	518669	<i>Rps</i> , Sclerotinia stem rot, and southern stem canker
Tokyo	548493	SMV-G1, SMV-G5, frog-eye, and northern stem canker
PI 54610	54610	SDS, frog-eye, and northern stem canker
Dunfield	548318	None found

<sup>1</sup> Source: Germplasm Resource Information Network (GRIN) at <https://npgsweb.ars-grin.gov/gringlobal>

<sup>2</sup> *Rps* = resistance to *Phytophthora sojae*; SMV-G1 = Soybean Mosaic Virus – G1; Soybean Mosaic Virus – G5; and SDS = sudden death syndrome

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