Doi: HTTPS://DOI.ORG/10.23910/2/2022.0455a

Black Root Rot of Strawberry: A Disease Complex

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Article History

Article ID: IJEP0455a
Received on 07th January, 2022
Received in revised form on 05th May, 2022
Accepted in final form on 21st May, 2022

Abstract

Historically, root diseases have been a production-limiting problem for the strawberry industry worldwide. Even though this disease is of great economic importance, the etiology remains unresolved. However, soilborne fungal root pathogens, particularly *Pythium* and *Rhizoctonia* spp. have been implicated as major role players. Presence of nematodes in soil and favourable environmental conditions also play significant role in the disease development. Development of integrated disease management strategies is dependent upon a more complete understanding of the etiology, biology and ecology of the disease complex.

Keywords: Crop rotation, biological control, etiology, disease complex

1. Introduction

Strawberry (*Fragaria×ananasa*), belonging to family Rosaceae, is a major fruit of temperate regions but the day-neutral cultivars can be grown profitably in subtropical areas (Hossain, 2007). Strawberry fruits are rich source of vitamin A, C, E, folic acid, calcium, polyphenols and phytosterols. It is also a good source of antioxidants (Anonymous, 2009). Two of the major hazards of strawberry production in mild tropics are diseases attacking fruits and plants and lack of superior day-neutral cultivars.

Strawberry suffers from a number of fungal and bacterial diseases. Black root rot (disease complex), grey mold rot (*Botrytis cinerea*), Rhizopus rot or leak rot (*Rhizopus stolonifer*), anthracnose (*Colletotrichum fragariae*), Verticillium wilt (*Verticillium dahlia*), red root rot or brown stele (*Phytophthora fragariae*), leather rot (*Phytophthora cactorum*) and powdery mildew (*Sphaerotheca macularis*) are some of the economically important diseases of strawberry (Murthy and Pramanick, 2014). Among different diseases, black root rot is a common, yield-limiting and serious disease complex that adversely affects strawberry production in many regions of the world. It is a widespread disease of strawberry that causes death of feeder roots and degradation of structural roots ultimately reducing productivity (Maas, 1998). This disease has been reported to cause losses in Australia (Porter et al., 1999), Western Cape Province (Botha et al., 2001), the Netherlands (Klinkenberg, 1955), Japan (Watanabe, 1977), the United Kingdom (Wardlaw, 1927) and the USA (Yuen et al., 1991; Duniway, 1998). It is a complex problem as the etiology of this disease is not clearly defined and varies amongst different locations. However, fungal pathogens have been reported as the primary cause of this disease (Ellis, 2000; Botha et al., 2001).

2. Symptomatology

Plants affected by black root rot are characterised by stunted growth, they wilt under heat stress and have brittle, blackened root systems (Strong and Strong, 1931; Wilhelm and Paulus, 1980). They also have fewer crowns of reduced diameter than unaffected plants and will produce less fruit of reduced quality. The leaves of the affected plants are typically smaller and fewer runners are produced (Elmer and LaMondia, 1999). Diseased plants show a loss in productivity and vigour due to the rotted roots. Black root rot of strawberry has often been associated with fields that have been in production for a long time (Ellis, 2000). The first symptoms are seen in only a few plants, mostly in areas where drainage is insufficient and where the soil has become compacted due to cultivation. When the stunted plants are removed from the soil, black root rot symptoms are seen showing blackened brittle, dead roots (Wing et al., 1995). Affected plants are devoid of feeder roots and many of the larger roots have broken off at rotted portions where the cortical tissue has collapsed, which gives broken off roots a “rats-tail” appearance. Symptoms are usually most prominent in the last few weeks before harvest. Some of the plants die during the production season because of root rot, but those that survive will be stunted and will produce a reduced crop of small strawberries. Replanting strawberries in fields previously cultivated with black root rot affected strawberries, is not successful and the disease incidence will increase significantly (Ellis, 2000).

3. Etiology

According to Strong and Strong (1931), black root rot of strawberries had been reported by various authors in the early 1900's, and was referred to as a disease condition of unknown etiology. In the 1920's, a number of investigations into the cause of the disease were carried out in different strawberry growing areas across the globe and various fungi and bacteria were implicated in the etiology. In the USA, Heald (1920) and Coons (1924) ascribed the disease to *Rhizoctonia.* In Scotland, Wardlaw (1927) implicated a species of *Pythium* and an unidentified sterile fungus in the cause of the disease. Plakidas (1930) also found a *Pythium* sp. to be highly pathogenic to strawberries in the USA. Although Berkeley and Jackson (1924) had implicated bacteria as causal agents, he was unable to confirm pathogenicity. In contrast to Berkeley and Jackson (1924), Strong and Strong (1931) were of the opinion that the disease was caused by *Coniothyrium fuckelii* Sacco and *Hainesia lythri* (Desm.) Hohn.

The possible role of nematodes in this complex was only realised in the late 1950's, when the root knot- or meadow-nematode (*Pratylenchus penetrans* Cobb.) was thought to be the causal agent of black root rot (Raski, 1956). Root feeding by this nematode caused stunting of plants and blackening of roots. Experiments conducted by Chen and Rich (1962) revealed that *P. penetrans* only predisposed plants to fungal infection by creating wounds. The idea that nematodes were the sole causal agents of black root rot was thereafter discarded.

3.1. Association of Pythium species with black root rot of strawberries

Since the 1960's, many fungi including species of *Pythium* have been implicated as the cause of the disease. In the USA, *Pythium sylvaticum* Campbell & Hendrix was most commonly isolated, occurring in 76% of the fields surveyed, but *P. irregulare* Buisman and *P. perniciosum* Serbinow were also frequently isolated (Nemec and Sanders, 1970). Pathogenicity tests confirmed that *P. sylvaticum* was pathogenic and Nemec (1970) subsequently proposed that it was the primary pathogen in the Illinois area. Other *Pythium* species that were isolated from the strawberry plants in the Illinois area included *P. dissotocum* Drechsler, *P. ultimum* Trow, *P. hypogynum* Middleton, *P. rostratum* Butler and *P. acanthicum* Drechsler. Denman (1994) made isolations from black root rot affected plants at the end of the 1993 season in the Western Cape, South Africa. A low level of *P. irregulare* was isolated from affected plants. In some studies on black root rot of strawberries, researchers in different areas of the world placed more emphasis on the role of *Rhizoctonia* spp. and *Pratylenchus penetrans* than on *Pythium* (Ribeiro and Black, 1971; Maas, 1984; Elmer and La Mondia, 1999).

3.2. Association of Pratylenchus penetrans with black root rot of strawberries

In the past, it was suggested that the root lesion nematode, *P. penetrans,* was the primary cause of black root rot disease (Klinkenberg, 1955; Raski, 1956). Townshend (1962) confirmed the pathogenicity of *P. penetrans* on strawberries in 1962. It was believed that nematodes provided soilborne fungi with an infection point through the wound they made when feeding (Chen and Rich, 1962). Plants growing in nematode infested soil showed root rot symptoms similar to those with black root rot (Goheen and Smith, 1956). It was thought that when the nematodes were not present, the fungi associated with black root rot could not infect and no disease would develop (Chen and Rich, 1962). However, Raski (1956) conducted experiments in soil from a black root rot infested strawberry field. This trial showed that *P. penetrans* could not cause black root rot without the presence of other pathogens implicated. However, nematodes do contribute to black root rot if they are present in soil along with other pathogens involved in the disease complex. Surveys were carried out using eight commercial strawberry cultivars that were planted in soil collected from strawberry rhizospheres at various locations in New Hampshire. It was evident from this survey that there was a definite association between *P. penetrans* and soilborne fungal pathogens forming part of the black root rot complex (Chen and Rich, 1962).

3.3. Association of Rhizoctonia species with black root rot of strawberries

The other organism that is most commonly associated with the black root rot complex is *Rhizoctonia.* This filamentous soilborne fungus survives long periods in soil by living on plant debris (Cotterill, 1993) or by forming sclerotia (Banniza et al., 1999). The genus *Rhizoctonia* consists of many hosts specific and non-specific, as well as pathogenic and non-pathogenic species. Since De Candole first described the genus *Rhizoctonia* in 1815, more than a hundred species have been placed in the genus (Ogoshi, 1987).

Rhizoctonia is mostly found as an imperfect basidiomycete fungus. Different species of *Rhizoctonia* have teleomorphs in different genera. For example, *Rhizoctonia solani* J.G. Kuhn has a *Thanatephorus* Donk teleomorph, while *Rhizoctonia fragariae* S.S. Husain & W.E. McKeen has a *Ceratobasidium* D.P. Rogers teleomorph. In general, mycelia of *Rhizoctonia* species exhibit extensive right angle branching near the distal septum. Clamp connections are not present, nor are conidia. A rhizomorph is not formed, and sclerotia are not differentiated into a rind and a medulla (Ogoshi, 1987). It has been recognized that there are great differences amongst the various isolates identified as *R. solani.* Differences in pathogenicity, sclerotial morphology, cultural appearance on media and other physiological characteristics are evident. These differences have been utilised to form a number of intraspecific groups or ISG's which assist in the identification of *R. solani.* However, the initial step in species identification of *Rhizoctonia* is to determine the nuclear status of the isolate. The genus *Rhizoctonia* contains isolates that either have multinucleate cells (and this is one of the main criteria for isolates to be classified as *Rhizoctonia solani*) or binucleate cells.

To determine the nuclear status of isolates, rapid and effective nuclear staining techniques are necessary. Many different nuclear staining methods are available. However, some of the methods are complicated and time consuming. For example, the HCL-Giemsa staining method was used frequently because of its reliability, and the distinctiveness of the stained nuclei, which made them easy to count (Herr, 1979). However, the process of fixation and acid hydrolysis is very time consuming and tedious. Trypan blue was used as an alternative to the HCL-Giemsa, but the trypan blue did not stain all the nuclei of all the isolates, neither did aniline blue. The acridine orange method proved to be excellent for quantifying nuclei in consecutive cells on specific strands of hyphae (Yamamoto and Uchida, 1982). The nuclei were not difficult to locate and the method has been proven reliable although one of the disadvantages of this method is the need for a fluorescence microscope. The safranin O-KOH method is equally rapid and can be used with bright field microscopy. The safranin apparently stains the nucleolus, rather than the nucleus (Yamamoto and Uchida, 1982). Both these methods have been proven useful in staining *Rhizoctonia* nuclei and are simple to use, rapid and give reliable results. Once the nuclei have been counted, the anastomosing group must be determined. Anastomosis is the process during which compatible hyphae from two different isolates fuse and exchange genetic material. The anastomosing group that an isolate belongs to is of importance because the morphology and virulence of different AG types within a species varies greatly (Muyolo et al., 1993). Eleven anastomosis groups have been identified and confirmed in *R. solani* based on hyphal anastomosis, cultural morphology, virulence, disease type and DNA base-sequence homology. All these factors (anastomosis group, pathogenicity, cultural morphology etc.) are important facets of identification as demonstrated by the AG-l group of *R. solani* which has recently been sub-divided into three groups AG-l IA, AG-l IB and AG-lIC (Muyolo et al., 1993).

Parmeter et al. (1967) found isolates of *Rhizoctonia* in strawberries that were similar to *R. solani* but had a *Cera tobasidium* teleomorph and not a *Thanatephorus* teleomorph. These *R. solani-like* isolates had predominantly binucleate hypal cells (Martin, 1988). Isolates of *Rhizoctonia* from California also had binucleate hyphal cells and were identified as *R. fragariae.* This led Parmeter et al. (1967) to confirm the identity of their isolates as *R. fragariae.*

Many other binucleate *Rhizoctonia* spp. have been identified in a range of hosts. Burpee et al. (1980) divided the binucleate group of *Rhizoctonia* species into seven different anastomosing groups (CAG-groups) and *R. fragariae* was placed in one of these groups, CAG 2. However, Ogoshi (1983) separated the binucleate *Rhizoctonia* spp. into at least 15 anastomosing groups (AG-groups) and three of these groups, *viz.* AGA, AGG, or AGI were associated with *R. fragariae.*

4. Epidemiology

Nemec (1970) demonstrated that soil moisture is critical for successful infection of strawberries by mycelia and germinating sporangia of *Pythium* spp. An abundance of soil water promoted germination of sporangia and production of zoospores as well as root infection. However, some researchers reported that *Pythium* was not able to penetrate the host directly but needed a wound or other opening to obtain access to host tissue (Adegbola and Hagendorn, 1969). Excessive irrigation favoured disease development by *P. sylvaticum* in California (Nemec, 1970) but, zoospore formation was not observed. *P. sylvaticum* was noted for its ability to colonize runner plant roots early in the growing season, which suggests that this pathogen either has a preference for young host tissue, specific nutritional requirements in the rhizosphere or requires an optimum temperature for infection to take place. It has been suggested that *Pythium* only plays a role in areas where there is enough free water in the soil and optimum temperature conditions (Nemec and Sanders, 1970).

In 1984, it was suggested that black root rot was not caused by one specific agent, but by a number of different factors. Although soilborne fungi and root-lesion nematodes were involved, other environmental effects such as freezing injury and water logging were also implicated (Maas, 1984). Environmental factors largely predisposed plants to infection (Wing et al., 1995) and agronomic practices often aggravated the situation.

Sandy soils with good drainage promoted the development of healthy roots, whereas soils with a high clay content and bad drainage supported poor root health and more severe disease development (Wing et al., 1995). Chemical components of soils did not have a major effect on the development of black root rot, but cultural practices did. Any factor that decreased the vigour of the roots increased the chance of black root rot developing.

Cultural practices that included, for example, high planting beds that caused shorter water saturation periods and allowed better oxygen concentration, led to plants that had better root health. It was also shown that if fields had been planted with strawberries within the previous five years, or had been used for strawberry cultivation for a number of years continuously, or if the current plantings were old, poor root health and black root rot symptoms were prevalent.

The repeated use of the herbicide terbacil (Sinbar, E.I. du Pont de Nemours and Co., Wilmington, Delaware) stressed plants, making them more susceptible to disease (Wing et al., 1995). Prevailing weather conditions throughout the season also affected plant health and the development of black root rot.

5. Management

5.1. Crop rotation

Practices such as crop rotation, ammonium sulphate [(NH4) S04] amendments and soil solarisation (Elmer and La Mondia, 1999) have been investigated and appear promising. It was found that a single rotation with Saia oats (*Avena strigosa* Schreb.) and the use of (NH4) S04 resulted in larger strawberries, a higher early yield and less damage from black root rot (Elmer and La Mondia, 1999). The addition of organic amendments such as biofumigants or organic composts has also been investigated, but the results have been variable and the structure and quality- of the soil greatly influence the efficacy of these amendments. Yields from organically treated soil have not been as good as those from chemically treated soils (Duniway, 1998).

Crop rotation has proven effective in many root and stem rotting diseases caused by *Pythium* spp. as well as *Rhizoctonia solani* and binucleate *Rhizoctonia* species including (*R. cerealis*) in crops such as cucumber, snap-beans, carrots, wheat and barley (Vilich 1993; Davis and Nunez 1999; Sumner et al., 1999). Crop rotation as a method of controlling of black root rot was investigated by Elmer and LaMondia (1999). Plots with a history of black root rot were seeded with sorgho-sudangrass (*Sorghum bicolor* (L.) Moench. x *S. sudanense* Stapf.) or Gary (*Avena sativa* L.) or Saia oats. Gary and Saia oats as well as Triple S sorgho-sudangrass were shown to be poor hosts for *Rhizoctonia fragariae* and *P. penetrans,* and suppressed disease. Other crops such as canola *(Brassica napus* L.) and buckwheat (*Fagopyrum escultentum* Moench.) did not have any effect on the population densities of *R. fragariae* and *P. penetrans,* or even increased the numbers of these pathogens in the soil (Elmer and LaMondia, 1999). Plots sown with Gary oats showed reduced levels of infestation with *R. fragariae* when compared with the controls. Rotation with grains, however, is not acceptable to the local strawberry fanners. Strawberries are grown on small areas that are not large enough to produce grains economically. Other crops need to be evaluated for use in a rotation plan with strawberries. The crop used has to be chosen and evaluated very carefully, because certain crops can increase the population densities of pathogens, and thus increase the disease intensity. The use of barley as a cover crop has shown to be very effective to reduce the pathogen levels in the soil (Vliet et al., 1996).

5.2. Resistant cultivars

Wing et al. (1995) evaluated resistance of twenty commercial strawberry cultivars to black root rot. Under the experimental conditions used in this trial none of the cultivars showed any resistance to this disease complex. Martin (2000) evaluated the growth and yield of 14 cultivars on non-fumigated, soil that had a history of black root rot. All cultivars except “Laguna” showed significantly reduced growth when compared to the control that was planted on methyl bromide + chloropicrin fumigated soil. Seven cultivars (Aromas, Capitola, Parisbad, Douglas, Gaviota, Languna and Parker) had yields that were more than 70% of that of the control plants (Martin, 2001). This was only a preliminary trial however; this is a crucial area of research that urgently needs to be addressed. It is essential to know and understand the etiology of black root rot in order to establish an effective resistance testing programme.

5.3. Biological control

Biological control through the addition of microorganisms to the rhizosphere, such as cyanobacteria, can be an effective means of control of certain soilborne diseases of strawberries. Many bacterial species are natural inhabitants of soil and some can fix nitrogen, thus enhancing soil fertility as well as controlling plant disease (Kulik, 1995). The uses of other fungi as control for pathogenic fungi have also been evaluated. *Rhizoctonia* AG-K induced resistance to *R. solani* AG 4 on soybeans and had the potential to be used as biocontrol agent.

Mycorrhizal fungi (*Glomus* spp.) were shown to increase the production of strawberry runners by up to 76% in the first year (Niemi and Vestberg, 1992).

5.4. Chemical control

Control of black root rot has relied mostly on the use of soil fumigants such as methyl bromide (Yuen et al., 1991; Elmer and LaMondia, 1999; Porter et al., 1999; Botha et al., 2001). Due to the imminent retraction of methyl bromide from use, an urgent need for alternative control measures has developed. There are a few different approaches to the solving of this problem (Denman and Botha, 1999). The one approach is to replace methyl bromide with different chemical fumigants such as chloropicrin and metham sodium. In Australia research has been carried out on the use of these alternative fumigants, and the combination of these fumigants with lower levels of methyl bromide, as a short-term solution to the problem (Porter et al., 1999). Long term solutions have not yet been developed, but the only plausible solution for the sustainable control of soilborne pathogens is in the form of an integrated control system (ICS) (Porter et al., 1999). This approach includes the use of biofumigants, biological control, crop rotation, nutrient management and resistant cultivars. In California the search for an alternative to methyl bromide has been carried out in two areas. Firstly, in the use of other chemical fumigants, and secondly in the use of non-chemical methods. With regard to the former, good results have been achieved by using 1,3 - Dichloropropene with chloropicrin and also with chloropicrin alone (Yeun et al, 1991). Vapam (methyl isothiocyanate) has also shown promising results (Duniway, 1998). Unfortunately, the use of chemical fumigants is not a long-term solution to the problem. Chloropicrin, commonly known as tear gas, is user- and environmentally-unfriendly and will probably not remain a viable option for much longer.

In South Africa there has not been any research done into alternatives to methyl bromide. Fungicides can be used as a short-term solution, but only a few are registered for use on strawberries in South Africa (Botha et al., 2001). Many problems are associated with the use of fungicides. If used too often, pathogens can become resistant to fungicides, rendering them ineffective. Fungicide treatments are not always economical and are not environmentally considerate. Another problem with using fungicides to control black root rot is the spectrum of pathogens that are involved in the disease complex. Fungicides target specific groups of pathogens but in this complex a variety of phylogenetically divergent pathogens are involved. Thus, one fungicide alone will not be effective for controlling black root rot. A combination of different fungicides will have to be used, and the right combinations have to be found.

Rhizoctonia is the one fungus that has been most consistently associated with the black root rot disease complex in most cases and areas. Pre-plant treatments of carboxin and iprodione have been recommended against *R. solani* and gave excellent results on *Brassica* species (Kataria et al., 1993). Other fungicides that have been used and recommended are PCNB (Pentachloronitrobenzene) and metalaxyl. PCNB controlled *Rhizoctonia*-induced rotting effectively on strawberries and metalaxyl *Pythium* induced diseases (Castillo and Peterson, 1990). It was shown that colonisation of alfalfa roots by *Pythium* or *Rhizoctonia* was also reduced by the use of these fungicides (Hancock, 1993). Fosetyl-Al controlled *Pythium*-induced diseases and can be used as an alternative to metalaxyl (Castillo and Peterson, 1990). Metalaxyl and fosetyl-Al are also used on strawberries for the control of certain fruit rots, such as leathery rot caused by *Phytophthora cactorum* Lebert & Cohn (Madden et al., 1991). In South Africa not many fungicides have been tested for use on strawberries and only captab and copper oxide are registered for use on this crop for Botrytis fruit rot and leaf spot.

6. Conclusion

The most effective, economical and environmentally safe control strategy for black root rot of strawberries is an Integrated Disease Management Program. The principles on which these programs rely are as follows: optimisation of pest control in an ecologically and economically sound manner; emphasis on co-ordinate use of multiple tactics to enhance stable crop production; and maintenance of pest damage below injurious levels while minimising hazards to humans, animals, plants, and the environment. The system includes chemical control, but it limits the use of chemicals by utilising other control measures that are available for use.

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