1 Introduction

The rose is a widely studied plant and much has been written about it. Without delving much in to the history suffice it to say that the wild roses are believed to have their origins in Europe, America, the orient and China, and in the Middle East (Beales 1997, Joyaux 2003). Rose cultivation dates way back to around 3000 B.C. in the ancient Chinese gardens (Beales 1997). Three important organizations are concerned with the formulation of classes and the nomenclature of roses: The American Rose society through their publications "Modern Roses", The World Federation of Roses Societies, and the British Association Representing Breeders (Baeles 1997). Beales (1997) has also given what he calls a simplified classification of roses based on their flower characteristics. The details of rose classification and the associated controversies are not the subject here, but it is important to mention that roses belong to the family Rosaceae, and Genus *Rosa*.

The rose has established itself as the most popular garden plant in the world as well as the most important commercial cut flower grown under glass (Horst 1983). Cut flowers contributed to 2,787 million of trade turnover in Europe in the year 2000 (Eurostat 2000). A fifth of the cut flowers were from none European countries and roses made 47 % of the imports from none European countries, which was worth 282 million Euros in the year 2000 (Eurostat 2000). According to the year 2000 statistical analysis the main destination for all cut flower imports into Europe was Netherlands but the highest consumer of cut flowers in Europe was Germany. The highest ranked exporters of cut flowers into the Europen Union (EU) were Kenya (25 %), Colombia (17 %) and Israel (16 %) to mention but a few (Eurostat 2000).

Despite the popularity of the rose, its cultivation is associated with various diseases. The most important rose diseases caused by fungal pathogens are: powdery mildew caused by *Sphaerotheca pannosa*, the blackspot disease by *Diplocarpon rosae*, and rust by *Phragmidium* species. The most important bacterial disease is the bacterial wilt caused by *Agrobacterium tumefaciens*.

The blackspot of roses is a foliar disease recognized by black spots on the upper side of the leaf. The symptoms are brown to black circular spots with an irregular margin on the upper surface of the leaf, which are followed by yellowing and premature defoliation. The disease is caused by a fungal pathogen with the perfect stage *Diplocarpon rosae* wolf (Wolf 1912) and the imperfect stage that is called *Marssonina rosae* (Lib.) Lind (Baker 1948). Other synonyms to the imperfect stage are *Asteroma rosae*, *Actinonema rosae*, and *Marsonina rosae* (Baker 1948, Horst 1983) among many others.

In temperate regions the first infections in spring are caused by the conidia or ascospores found in the overwintered plant material. The fungus overwinters on dormant stems, thorns, fallen leaves, and buds (Lyle 1943, Cook 1981). The conidia are spread by wind, rain splash and probably by animal vectors including insects and arachnids (Palmer et al 1978). Records of the apothecia containing eight ascospores are rare and the importance of the genetic variation generated by sexual recombination is not clear (Walker 1995). The formation of apothecia has been reported in North America (Wolf 1912, Aronescu 1934), Great Britain (Knight and Wheeler 1977, Cook 1981) and in Russia (Dudin 1972).

The disease was first described in Europe in Sweden in 1815, then in Belgium in 1827 and later in other European countries (Baker 1948). The first report in North American were in 1831, South America in 1880, Australia 1892, Africa in 1920, and in China in 1910 as summarized by Baker (1948). Today the disease has a worldwide distribution, even occurring on oceanic islands like the Philippines and Hawaii (Horst 1983, Drewes-Alvarez 2003).

Blackspot of roses is an important, devastating and widespread disease. In out door growing of roses black spot disease is generally present, often as a major problem, and frequently in epidemic proportions (Horst 1983). It is a minor problem in greenhouse roses because humidity is regulated very carefully but is the most important disease of out door roses (Horst 1995). The rose plant is used in gardens and landscaping for its aesthetic value, but the blackspot infections make the roses unsightly due to the black spots on the leaves, yellowing and premature defoliation. The pathogen causes defoliation and weakening of the plants (Drewes-Alvarez 2003). The premature defoliation leads to reduced vigour (Smith et al 1988) and even death in very susceptible varieties (Black et al 1994). The damage caused by the *D. rosae* is greater than the leaf spots because of the added effect of the premature defoliation. Disease damage cannot be assessed only in terms of size of lesion but always includes the defoliation aspect.

Symptom expression in the blackspot disease has been reported to differ with pathogenic races, varieties, and environmental conditions. Rose cultivars differ in their susceptibility to *D. rosae* and pathogenic races of this pathogen have been reported by several authors (Debener et al 1998, Malek et al 1996, Bolton and Svejda 1979, Jenkins 1955, Knight and Wheeler 1978b). In a study using a set of differentials of rose cultivars, Malek et al (1996) identified at least five physiological races of *D. rosae*.

The widely cultivated tea hybrids and floribunda roses are susceptible to the blackspot pathogen (Reddy et al 1992, Walker et al 1995). Some of the wild species of roses have resistance but they lack the desired aesthetic traits (Reddy et al 1992). High resistance to blackspot has been found in genotypes of *R. banksiae* Ait, *R. carolina* L., *R. laevigata* Michx., *R. multiflora* Thunb. Ex Murray, *R. rugosa* Thunb., *R. roxburghii* Trait., *R. virginiana* Herrm. and *R. wichurana* Crèp (Drewes-Alvarez 2003). The resistance mechanism in roses is thought to operate through mechanical resistance of the cuticle (Castledine et al 1981), diffusible substances (Walker et al 1995, Knight and Wheeler 1978) or to be a post penetration defence response (Wiggers et al 1997). Debener (2003) reported that in a recent screening of tetraploids and diploids carrying resistance from *R. multiflora* with single conidial isolates the presence of two dominant genes Rdr1 and Rdr2 was revealed, but this does not in any way rule out horizontal resistance.

The resistance found in the wild roses needs to be transferred into the modern cultivars. Attempts to transfer resistance from the wild species to the modern roses by introgressive breeding led to a resistant F1 hybrids but the resistant was lost in the first backcross maybe due to lack of pairing partner at meiosis (Walker et al 1995). The integration of resistance genes by crosses with wild species is complicated due to varying ploidy levels in rose species and possible linkage of resistance genes with undesirable traits (Dohm et al 1996). In addition to that, breeding of rose cultivars that are resistant to blackspot is very time consuming because of the generative reproduction and complex genetic constitution of roses (Malek et al 1996). Research for the development of resistance against the disease are still under way (Debener et al 1996, Debener 1996), but the control of black spot still requires intensive use of systemic fungicides (Walker et al 1995, Reddy et al 1992).

Once established on plants, blackspot is difficult to control despite a combination of practices that include sanitation measures and fungicide applications (Behe et al 1993). Good cultural practices include: Removing disease leaves from the ground and pruning canes with infected leaves to reduce the overwintering potential of the pathogen; avoiding dense planting to allow good air circulation through the leaf canopy (Horst 1983); avoiding overhead irrigation since it favours infection; avoiding excessive watering during dark and humid weather; avoiding keeping the rose leaves wet for long hours, as this provides the water needed for the conidia to germinate.

In general, a good understanding of a pathogen's life cycle is a prerequisite to its effective control. Despite several research contributions to the understanding of the development of *D. rosae* in roses, there are still discrepancies in the observations made on its life cycle. The existing histological studies of *D. rosae* (Aronescu 1934, Frick 1944 and Palmer et al 1978) were limited by the scope of the then available techniques and equipment. Aronescu (1934) and Frick (1944) made drawings of their microscopic observations, while Palmer et al (1978) commented on the inability to make better photographs due to poor sectioning apparatus. There are no detailed ultra structure studies of the infection process of *D. rosae* on roses. Therefore, there is need to have a detailed and more accurate documentation of the infection process of *D. rosae* using modern technology and there is need to shed more light into the discrepancies in the life cycle of this pathogen.

Given that the management of *D. rosae* heavily relies on chemical control, there is need to keep on testing effectiveness of new fungicides active ingredients in controlling this pathogen. Due to the increase in the general environmental protection awareness world wide, the need to replace the fungicides in the market with more environmentally friendly ones has never been more timely. This can be achieved through testing of new more environmental friendly fungicidal active ingredients with the hope of offering better disease management.

The conidia of the blackspot pathogen germinate to form germ tubes on the host surface but further development of the fungus takes place below the host cuticle and even within the host cells. The control of this type of pathogen requires the use of contact as well as systemic fungicides. Couch (1995) distinguishes fungicides by their topical or physical mode of action: contact fungicides stay outside of plants, and penetrants penetrate the plant in some manner. Localized penetrants diffuse into leaf surfaces. Acropetal penetrants are transported by xylem towards the leaf tips after they penetrate (upward movement) but do not move in the phloem towards the root tips. Systemic penetrants are fungicides that are transported by both xylem and phloem. Penetrant fungicides in general offer the possibility of prolonging disease control, as the fungicide protects plant surfaces, but they also may inhibit pathogens in the early stages of infection inside the plant.

One group of fungicides that have been widely used for the control of many leaf spot pathogens including *D. rosae* are the demethylation inhibitors or DMI fungicides. This is a homogenous group of fungicides showing a common mode of action within the fungal sterol biosynthesis pathway i.e. the inhibition of demethylation at position 14 of lanosterol or 24-methylene dihydrolanosterol, which are precursors of sterols in fungi (Kuck et al 1996). Azoles such as imidazole and triazoles derivatives are the most important DMI fungicides. DMIs have a broad spectrum of fungicidal activity with most fungi from the Ascomycetes, Basidiomycetes and Fungi Imperfecti. As reported by Kuck et al (1996), a multitude of leaf spot pathogens are effectively controlled by several DMIs. According to the report of Newmann and Jacob (1996), all DMIs are able to penetrate the plant cuticle and /or seed coat to some extent and they are then translocated in either the apoplast (transport in coherent network of free space, cell walls and non-living cells) or symplast (transport in the coherent network of protoplasts connected by plasmodesmata). DMIs have minimal symplastic/basipetal movement i.e. movement in the living part of the plant which is the phloem and cytoplasm, but their translocation is predominantly apoplastic and protection is confined to such cases where sufficient quantities of active ingredients have been applied to the basal parts of the leaf or shoot (Kuck et al 1996). The DMIs of interest in this study are two triazoles, namely myclobutanil and tebuconazole.

A new group of fungicides with much promise in the control of a wide range of fungal pathogens are the strobilurins. Strobilurin fungicides were developed following the discovery of naturally occurring β-methoxyacrylates, the simplest being strobilurin A and oudemansin A produced naturally in certain species of mushroom fungi, including

Strobilurus tenacellus and *Oudesmansiella mucida* (Ypema and Gold 1999). These fungicides are now more properly referred to as Q_oI. Industrial chemists improved these natural fungicides by making chemical modifications that resulted in compounds that were less subject to break down on the leaf surface by sunlight. More strobilurins continue to be released in the market. Some of the fungicides active ingredients found in this group include trifloxystrobin, pyraclostrobin, kresoxim-methyl and azoxystrobin (Bartlett et al 2001).

As reported by Sauter et al (1995), all Q_oI fungicides share a common biochemical mode of action: they all interfere with energy production in the fungal cell. They block the electron transfer at the site of quinol oxidation (the Qo site) in the cytochrome bc_1 complex, thus preventing ATP formation. They are site-specific fungicides.

Ebeling (2003) reporting on one of these active ingredients, namely trifloxystrobin, said that it has a very favourable profile concerning toxicology, residue behaviour, environmental fate and ecotoxicology. It has low acute toxicity, no likelihood of causing acute hazards, no genotoxic or carcinogenic potential and also no signs of neurotoxicity. The active ingredient rapidly degrades in the environment and no risk to the consumer is expected by the way of residue intake through food.

With important exceptions, the Q_oI control an unusually wide array of fungal diseases, including diseases caused by water molds, downy mildews, powdery mildews, leaf spotting and blight fungi, fruit rotters, and rusts. They are used on a wide variety of crops, including cereals, field crops, fruits, tree nuts, vegetables, turf grasses and ornamentals (Stark-Urnau et al 1997, Gold and Leinhos 1995). However, the effectiveness of these new fungicide active ingredients on the fungal structures formed by *D. rosae* has not been studied in detail.

This study was undertaken with the main aim of carrying out a detailed study of the life cycle of *Diplocarpon rosae* in roses leaves in order to clarify the discrepancies found in literature. The objectives also included an investigation into the state and the plant parts in which the fungus overwinters in the weather conditions in Germany. The study targeted at assessing the aggressiveness of *D. rosae* isolates from Kenya and Germany by visual observation of the isolates that led to the earlier development of disease symptoms, and through biochemical assays of relative accumulation of reactive oxygen species (ROS) in leaves of plants inoculated with different isolates. The objectives encompassed a disease control aspect in which the effectiveness of the novel fungicide active ingredients, the strobilurins, on the fungal structures within the host tissues at different developmental stages was compared with the effectiveness of the fungicide active ingredients already in the market.