Review of Literature

The present literature review mainly concern with host and pathogenic constitutive and/or induced factors that are responsible for causing SER disease in pineapple by *Ceratocystis paradoxa* and other fungal diseases only.

Pineapple Fruit

Pineapple is a non-climacteric tropical fruit. The cultivated pineapple (*Ananas comosus* (L.) Merrill, belongs to the family *Bromeliaceae*. Any reference to pineapple in this document refers to *Ananas comosus* var. Queen. The pineapple is the leading edible member of *Bromeliaceae* which consists about 2,000 species, mostly epiphytic and many strikingly ornamental. The pineapple shares the distinction accorded to all major food plants of the world of having been selected, developed, and domesticated by peoples of prehistoric times and passed on to us through earlier civilizations.

Pineapple is the 3rd largest fruit produced India next to Mango and banana. The total production of pineapple is around 14 million tons. In India, pineapple is produced in the state of Bihar, Kerala, West Bengal, Andhra Pradesh, Karnataka, Goa, Tamil Nadu, Orissa and North Eastern States. North Eastern states produces major share of pineapples
in India. Even though India is one of the major producers of fresh pineapples its position in world export is significantly low. Less than 2% of total production is used for processing. Among the various reasons, lack of proper post harvest handling, Infrastructure, and storage facilities crowned with heavy post harvest spoilage mainly stem end rot (SER), which affect long distance export of pineapple fruits in India. In developing countries like India, pineapple losses can be as high as 70% (Salunkhe et al., 1991).

**Systemic classification:**

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<th>Kingdom</th>
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Pineapple is a perennial monocotyledonous plant having a terminal inflorescence and a terminal multiple fruit. It continues to grow after fruiting by means of one or more axillary buds growing into vegetative branches with a new apical meristem. The main morphological structures of the plant are the stem, the leaves, the peduncle (stem which bears fruit), the multiple fruit or syncarp or sorosis (fusion of many fleshy fruitlets), the crown, the shoots and the adventitious roots (Coppens d’Eeckenbrugge and Leal, 2003). The inflorescence consists of 50-200 individual flowers, capped by a crown composed of
numerous short leaves (up to 150) on a short stem. *A. comosus* var. *comosus* flowers are normally self-sterile and fruit development is parthenocarpic (does not require fertilisation) (Py *et al*, 1987). The edible part of the fruit consists mainly of the ovaries, of the bases of sepals and bracts and of the cortex of the axis. The fruit shell is mainly composed of sepal and bract tissues and the apices of the ovaries (Okimoto, 1948).

**Maturity Index and harvest**

**Maturity**

The stage of maturity, method of harvest, handling and packing are largely determined by the ultimate destination of the fruit. It was revealed during the survey that adequate attention is not paid by the growers to harvest the fruit at the proper stage of maturity and both ripe and unripe fruits are indiscriminately gathered during harvest.

Pineapple undergo changes during the development, where the immature eyes are gray or light green and the small brackets or bracts which cover half of each eye are gray giving the fruit grayish appearance. As the fruit matures, the space between the eyes fills out and the colour gradually changes from light to dark green that indicate complete maturation of fruit (physiological maturity- referred as 80% maturity). As the fruit ripens, the eyes changes from pointed to flat with slight hollowness at the centre, the fruit becomes enlarged, less firm and more aromatic. The fruit is picked for domestic use when the fruit attained the yellowing (Harvest maturity-referred as 100% maturity). At this stage of maturity, the fruit has higher T.S.S. and low acidity. When the growing areas are far away from the market, the fruit is manually harvested at 80% maturity stage. Thus it takes 2-3 weeks before fruits are fully ripe. The growths of the ridge on the margin of the eyes are added indices for its harvesting if shipped for long distances For fruits intended for processing located near growing areas may be delayed till more colour develops on
the fruit. Change in surface colour that begins at peduncle end and progress towards
crown region of the fruit, Based on which maturity/ripe stages are categorized into
Green, 25%, 50%, 75% and 100% ripe (Collins 1968)

**Storage of pineapple**

Pineapple is a non climacteric, chilling sensitive fruit. Fruits tend to develop
chilling injury symptoms if stored below 10°C. The susceptibleness is depending upon
maturity stage at harvest, cultivar and storage condition. Matured Pineapple var. queen is
successfully stored at 12±1°C and 90±5% RH for 16 days with optimum ripening and
eating qualities. But these conditions fail to stop the *C. paradoxa* infection among field
infected fruits. The SER infection slows down in lower temperature but continues to grow
internally. SER disease of pineapple forms the major limiting factor during storage.

**Pathology of Pineapple fruit**

The present review focused on fungal diseases of pineapple fruit diseases
exhibited following harvest. Stem end rot disease caused by *C. paradoxa* is of
commercial importance. Many fruit disease symptoms have been described on pineapple.
In addition or a few, specific pathogens followed by massive infection by broader
spectrum secondary invaders are also reported (Collins, 1968; Rohrbach and Apt, 1986).
The bacterial diseases and physiological disorders were not presented in this review, since
it is out of scope of the present investigation.
Stem end rot (SER)

Stem end rot (SER), also called Black rot, *Thielaviopsis* fruit rot, water blister, soft rot or water rot, is caused by the fungus *Ceratocystis paradoxa* (De Seynes) Sacc. (syn. *Thielaviopsis paradoxa* (De Seyn.) Hohn *telemorph* Chalara paradoxa (Dade) C. Moreau). Phylogenetic and taxonomic evaluation of the pathogen Chalara paradoxa (De Seyen.) Sacc. (syn.) *Thielaviopsis paradoxa* (De Seyen.) Höhn., teleomorph: Ceratocystis paradoxa (Dade) C. Moreau has been conducted by Paulin-Mahady et al. (2002). The disease is a universal fresh-fruit problem. The severity of the problem is dependent on the degree of bruising or wounding during harvesting and packing, the level of inoculum on the fruit and the storage temperature during transportation and marketing. Black rot does not occur in the field unless fruit is overripe or injured. The pathogen gains entry into host tissue through wounds and causes black rot disease in pineapple (Rohrbach and Phillips, 1990). Black rot of the pineapple fruit is characterized by a soft watery rot, which usually starts at the point of detachment of the fruit. Diseased tissue turns dark in the later stages of the disease because of the dark colored mycelium and chlamydospores.

Infection of the pineapple fruit occurs through wounds resulting from harvesting and postharvest handling. Susceptibility Under conditions of high humidity, conidia may readily be produced on pineapple residue and be disseminated by wind to the unharvested fruit. Inoculum levels on fruit at harvest vary according to the environmental conditions prior to harvest (Rohrbach and Schmitt, 1994). The high correlation between moisture (rainfall duration) prior to harvest and disease following harvest has resulted in the name ‘water rot’. Infection occurs within 8–12 h following wounding. Refrigeration at 9°C during transportation will slow development of the
disease, but, when fruit are returned to ambient temperatures, disease development will resume (Rohrbach and Phillips, 1990). Fruitlet core rots (black spot) FCR (Oxenham, 1962; Rohrbach and Apt, 1986) or black spot (Keetch, 1977) (also called fruitlet brown rot and eye rot (Snowdon, 1990)) is a descriptive term for a brown to black colour of the central part of an individual fruitlet.

**Fruitlet core rots (black spot)**

Each major pineapple production area appears to have characteristic pathogens associated with the FCR symptom, probably as a result of the environmental conditions of the area (Rohrbach, 1980). For example, in Hawaii, Penicillium and Fusarium species are most commonly associated with FCR (Rohrbach and Apt, 1986). In South Africa, Penicillium species are most commonly found (Keetch, 1977), while, in Brazil, Fusarium species are most commonly associated with the FCR symptom (Bolkan et al., 1979).

FCR (Oxenham, 1962; Rohrbach and Apt, 1986) or black spot (Keetch, 1977) (also called fruitlet brown rot and eye rot (Snowdon, 1990)) is a descriptive term for a brown to black colour of the central part of an individual fruitlet. FCR is caused by an infection by a pathogen or, more commonly, a group of pathogens. Botanically the central area of the fruitlet core is the septa (inverted Y) between the three seed cavities or locules. Because individual or mixtures of pathogens may be associated with the FCR symptom, there is considerable confusion in the literature. The Penicillium and Fusarium fungi (Rohrbach and Apt, 1986), the round yeasts and bacteria.

The degree to which these symptoms develop appears to depend on the time of infection, the pathogen or mixture of pathogens present, the cultivar and environmental conditions. The IFC symptom can also be caused by boron deficiency in which case the symptoms are indistinguishable.
It is theorized that the very low levels are the result of botanical malformations of individual fruitlets caused by disruptions in the normal phyllotaxis of the fruit (Kerns et al., 1936). Malformation of the fruitlet allows infection of the stylar canals and nectary ducts by a range of pathogens. In contrast, true epidemics result from the coincidence of optimum environmental conditions resulting in predisposed flowers, production of inoculum of the pathogen(s) and transport of the inoculum to potential infection sites. The disease could become more important if some of the more susceptible, low-acid ‘Smooth Cayenne’ cultivars and hybrid cultivars are grown commercially for fresh-fruit markets. The FCR symptom is generally characterized by browning of the inverted ‘Y’ tissues.

As mentioned previously, the FCR complex involves the fungi P. funiculosum and F. subglutinans, and the round yeast Candida guillermondii. The pineapple tarsonemid mite, S. ananas, and the pineapple red mite, D. floridanus, are also associated with the FCR complex. Considerable information is known and published on the Penicillium- and Fusarium-induced fruit diseases and the role of the pineapple tarsonemid mite (Rohrbach and Pfeiffer, 1976b; Rohrbach and Taniguchi, 1984). FCR symptoms produced by Penicillium species are dark to medium brown in colour, usually with a grey, water-soaked centre FCR symptoms from yeast infections are usually light brown.

**Fusariosis**

Fusariosis is caused by the fungus *F. subglutinans* which is the conidial stage of *G. fujikuroi* Edwards. Whether or not *F. subglutinans* in Brazil is the same as the Fusarium species causing FCR is not definitive in the literature. Laville (1980) considers *F. subglutinans* a distinctly different species from the Fusarium causing FCR. Other authors have attributed FCR to *F. moniliforme* (Oxenham, 1962; Guerout, 1974;
Rohrbach, 1983). The disease, first described in Argentina in 1954, was first reported in Brazil in 1964 and within 10 years had spread over the entire country (Laville, 1980; Rohrbach, 1983). The fruit symptoms at low severity levels are similar to those of FCR, which vary from light through medium to dark brown, extending partially to completely down the fruitlet core. FCR from Fusarium sp. is usually a ‘dry’ type of rot. In Brazil, the symptom is not limited to a single infected fruitlet, as in typical FCR reported in other pineapple production areas. Fruit symptoms involve multiple fruitlets, with the infected area of the fruit surface appearing off-colour initially and later becoming sunken, with profuse pink sporulation and exudation of gum. Gum exudation can be confused with the exudation from Thecla wounds (Laville, 1980).

Optimum temperatures for growth are 25°C, with a range of 5°C to 35°C (Camargo and Camargo, 1974). Control of fusariosis is most effective by planting disease-free seed material and by controlling insects, particularly the bud moth (Laville, 1980). Fungicides, such as captan at 700, starting at differentiation through harvest at 20-day intervals, have given good control of the fruit-rot phase in Brazil (Bolkar et al., 1978).

**Miscellaneous fruit rots**

Fruit rots caused by *Aspergillus flavus*, *Botryodiplodia theobromae* and *Rhizopus oryzae*. Internal browning is a physiological disorder caused by chilling injury. Pests, Diseases and Weeds 241 or Rhizopus stolonifer have been reported as postharvest diseases (Snowdon, 1990). A fruit rot caused by *Hendersonula toruloidea* (Natt.) has been reported by Lim (1985) on the Mauritius cultivar. Green fruit rot caused by *Phytophthora* species occasionally causes large losses of lodged first-ratoon fruit in
Australia under very wet conditions. These pathogens generally require some form of wounding for infection. Commercially, these diseases are of very minor importance.

**Biochemical changes in Pineapple fruit during ripening and pathogenesis**

**Polyphenol oxidase (PPO)**

However, the browning after mechanical or physiological injury, suffered during either harvest or cold storage, and during *C. paradoxa* infection affects consumer acceptability and palatability. Most browning in fruits is caused by enzymatic oxidation of natural phenolic compounds. Polyphenol oxidase (EC 1.10.3.1; PPO) is the major enzyme that catalyzes the oxidation of phenolic compounds to quinones, which further polymerize to brown pigments (Lee, 1991). The pineapple PPO was found in three isoforms (Das, Boht, & Eowda, 1997), with the major isoform being a tetramer of identical subunits of 25 kDa and having optimum activity between pH 6 and 7. The PPO is stable to heat when extracted, but loses over 50% of its activity following 20 min exposure to 60 °C in vivo (Teisson, 1977). PPO characteristics have been thoroughly investigated in apple (Janovitz-Klapp et al., 1989), grape (Sánchez-Ferrer et al., 1988), potato, and mushroom (Chen et al., 1992). Although a number of studies on the morphological characteristics and physical and biochemical changes during pineapple fruit development are available (Gortner and Singleton, 1965; Kermasha et al., 1987; Bartolome et al., 1995), there is little or no information on PPO. In order to understand the role of PPO in the browning of pineapple fruit during cold storage, purification of PPO from pineapple was attempted.

Polyphenol oxidase (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase, EC 1.14.18.1) is an oxidative enzyme widely distributed in the plant kingdom and has been detected in most fruits and vegetables. PPO has been partially
purified from many fruits, including grape (Sanchez-Ferrer et al., 1989), apple (Oktay et al., 1995), guava (Augustin et al., 1985), peach (Laveda et al., 2000), banana (Sojo et al., 1998), pear (Siddiq et al., 1994), kiwi (Park & Luch, 1985), strawberry (Ebeling & Montgomery, 1990), plum (Siddiq et al., 1992), cherry (Pifferi & Culbera, 1974), and pineapple (Das et al., 1996). The localization of the enzyme in the plant cell depends upon the species, age, and in fruits and vegetables – on maturity. In potato tubers nearly all-sub cellular fractions were found to contain PPO. In freshly harvested apples, the enzyme is localized almost exclusively in chloroplasts and mitochondria (Vamos-Vigyazo, 1981). Where as its substrates (phenols) are localized in the vacuoles.

PPO is a copper-containing enzyme, which catalyses two entirely different reactions (a) the hydroxylation of monophenols to the corresponding o-dihydroxy compounds; (b) The oxidation of o-dihydroxy phenols to o-quinones. The reactions require molecular oxygen. The quinones formed from above reactions are very unstable and rapidly react with amino acids or proteins, generating brown pigments by polymerization (García-Carmona et al., 1988). These reactions are more important in fruits with high phenol contents such as eggplant, apple, potato (Sakamura & Obata, 1963; Bajaj et al., 1979). In the healthy cellular system PPO is separated from its substrates due to membrane compartmentation. Upon the loss of membrane integrity due to ripening/senescence/physical injury in the cells of fruit and vegetables, the contact of the enzyme and its substrates initiates browning reactions (Moskowitz and Hrdzina, 1981; Mayer, 1987).

Techniques have been developed to prevent browning and PPO activity, each requiring a different approach depending on the characteristics of the plant tissue and the PPO (Martínez and Whitaker, 1995; Walker and Ferrar, 1995). Post harvest treatments such as heat, waxing, atmosphere control and application of 1-methylcyclopropene
(Rohrbach and Paull, 1982, Selvarajah and Herath, 1997 and Selvarajah et al., 2001) have been tested as alternatives to prevent internal browning without success. In general, exposure of PPO to temperatures of 70–90 °C destroys their catalytic activity (Vamos-Vigyazo, 1981). Thermal inactivation profiles of PPO in fruit and vegetable processing follow first-order reaction kinetics with the time required varying with the product. Of the studies on heat inactivation of PPO only a few have included the calculations of Arrhenius and the kinetic parameters of heat inactivation of PPO from various foods. These include apple (Strübi, Escher, & Neukom, 1975), Sultana grapes (Aquilera, Oppermann, & Sanchez, 1987), apricot (Heil, McCarthy, & Merson, 1988), rice (Ansah, 1989) and mango (Askar, El-Ashwah, Omran, & Labib, 1994). No information is available for pineapple browning of tissue due to C. paradoxa infection, on the quantitative effects of temperature and time on the inactivation of PPO.

**Peroxidase (POD)**

Peroxidase (donor: hydrogen peroxide oxidoreductase, E.C.1.11.1.7) are, similar to PPO, member of the group of oxidoreductases and both enzymes catalyse more than one reaction and acts on a great number of substrates; Both are involved in enzymatic browning of fruit and vegetables (Williams et al., 1985; Nicolas et al., 1994). In plant cells POD is located in cytoplasm as a soluble form, and partly cell wall bound, which is in insoluble form, (Vamos-Vigyazo, 1981). Plant peroxidase is an iron-containing enzyme, which catalyses four types of reactions: (1) peroxidatic, (2) oxidatic, (3) catalatic, and (4) hydroxylation. It degrades hydrogen peroxide in the presence of a hydrogen donor. POD is highly specific to peroxide substrate, where as it has very low specificity for the hydrogen donor substrate. It uses wide variety of hydrogen donor substrates to decompose hydrogen peroxide. It can oxidize phenols to quinones, and then
condense tannins to brown polymers in the presence of H$_2$O$_2$, which may then contribute to enzymatic browning (Robinson, 1991). Increased POD activity has been observed in pineapple upon exposure to ozone, pollution, nutritional disorders, wounding, and chilling injury (Campa, 1991). The browning of pineapple and litchi fruit has been attributed to the POD activity (Zhang et al., 2005).

**Phenyl alanine lyase (PAL)**

Being a key enzyme in phenolic biosynthesis, phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) has also been considered to be associated with browning and the accumulation of chlorogenic acid and lignin-like materials (Hahlbrock & Scheel, 1989). PAL has been reported to play an important role in the browning process of many fruits and vegetables (Ke & Saltveit, 1989). During infection process of pathogens, the PAL activity was increased in order to render resistance to invading pathogen by excessive synthesis of polyphenols. Further oxidation reaction of these polyphenols leads to browning and cell death (Saltveit, 2000). Thus browning mechanisms in fruit tissues may involve any one of the above phenomenons or many interlinked phenomena. Different fruits have showed different mechanism of browning.

Blackheart development in pineapple fruit (*Ananas comosus*, Smooth Cayenne) has been attributed to activity of PPO, peroxidase and phenylalanine ammonia-lyase (Zhou et al., 2003). For example phenolics, PPO, PAL, and iron play important role in blackening reactions of stored artichoke heads (Lattanzio, et al. 1994), whereas PPO, peroxidase and anthocyanase enzymes together play important role in browning of litchi fruit (Zhang et al., 2005).
**Bromelain**

Bromelain is a crude, aqueous extract from the stems and immature fruits of pineapples (*Ananas comosus* Merr., mainly var. Cayenne from the family of bromeliaceae), constituting an unusually complex mixture of different thiol-endopeptidases and other not yet completely characterized components such as phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates, among others [Cooreman (1978); Rowan and Buttle (1994)]. In addition, bromelain contains several proteinase inhibitors [Lenarcic, et al., (1992), Hatano, et al., (1996)]. Stem-bromelain (EC. 3.4.22.32) is distinguished from fruit-bromelain (EC. 3.4.22.33), previously called bromelin [Rowan and Buttle (1994)]. Today bromelain is prepared from cooled pineapple juice by centrifugation, ultrafiltration and lyophilization. The process yields a yellowish powder, the enzyme activity of which is determined with different substrates such as casein (FIP units), gelatine (gelatine digestion units) or chromogenic tripeptides [Filipova, et al., (1984) Harrach, et al., 1995]. Bromelain contains several distinct cysteine proteinases that have similar but distinct amino acid sequences, as well as differences in proteolytic specificity and sensitivity to inactivation. Stem bromelain (EC 3.4.22.32, formerly EC 3.4.22.4) is the most abundant proteinase within bromelain preparations derived from pineapple stem. Other proteinases that are present at lesser amounts include fruit bromelain (the major proteinase present in pineapple fruit; EC3.4.22.33, formerly EC 3.4.22.4 and 3.4.22.5) and ananain (EC 3.4.22.31). Stem bromelain preferentially cleaves the Z-Arg-Arg model substrate, whereas fruit bromelain and ananain show minimal activity against this substrate. In contrast, fruit bromelain and ananain but not stem bromelain efficiently cleave the Bz-Phe-Val-Arg substrate (Laura, et al., 2005).
By high-resolution fast protein liquid chromatography (FPLC) and other biochemical methods, basic (stem bromelain, ananain, comosain) and acidic thiol-proteinases have been isolated from crude bromelain, partially or fully sequenced and characterized in more detail [Harrach, et al., (1995); Harrach, et al., (1998); Napper, et al (1994)]. They mainly comprise glycosylated multiple enzyme species of the papain superfamily with different proteolytic activities, molecular masses between 20 and 31 kDa, and isoelectric points between > 10 and 4.8. Two major basic proteinases, F4 and F5, were further characterized and showed molecular masses of 24,397 and 24,472 Da, respectively [Harrach, et al., 1995] These enzymes also differ in their susceptibility to inactivation.

**Enzyme Activities**

The activities comprise a wide spectrum with pH optima between 5.5 and 8.0 [Yoshioka, et al., 1991]. The substrate spectrum is similarly broad, extending from synthetic low molecular mass amides and dipeptides up to high molecular substrates such as fibrin, albumin, casein, angiotensin II, bradykinin. Bromelain preferentially cleaves glycyl, alanyl and leucyl bonds. Commercial bromelain preparations are evaluated according to their proteolytic activity. The platelet aggregation inhibitory and anti-inflammatory action seems to be related to the protease activity. However, other effects such as inhibition of tumor cell growth and metastasis as well as debridement of burns are associated with other nonproteinolytic components contained in bromelain. Thus, the determination of the proteolytic activity alone may not be sufficient to completely characterize the pharmacological properties of bromelain [Taussig and Batkin (1988)].

Ananain is reported to be rapidly inactivated by the chicken egg white proteinase inhibitor cystatin and by the suicide substrate, E-64 (trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane), but these inhibitors either very slowly or only
minimally inactivate stem and fruit bromelain (Laura, et al., 2005). In aqueous solution, bromelain rapidly deteriorates through self-digestion. The addition of serum containing a2-macroglobulin will prevent self-digestion.

**Organic acids**

Fruit acidity and sweetness are two of the major factors that determine pineapple fruit eating quality. Other measures of fruit quality include shell color, fruit size and shape, aroma, crown size, crown to fruit ratio and the absence of disease and blemishes (Paull and Chen, 2003). Variation of pineapple fruit acidity and sweetness are associated with the pineapple clone used, fruit maturation and growing conditions (Singleton and Gortner, 1965, Py et al., 1987 and Bartolome et al., 1995).

**Acidity**

Fruit acidity increases during pineapple fruit growth and as the fruit approaches maturity and starts to ripen, the acidity declines (Singleton and Gortner, 1965 and Smith, 1988). Citric acid shows the greatest changes during fruit growth, increasing and then reaching a peak prior to ripening, whereas malic acid shows little change during development (Singleton and Gortner, 1965 and Chan et al., 1973). Fruit sweetness gradually increases during the later stages of fruit growth (Bartholomew and Paull, 1986) with the sugar to acid ratio being recommended as a harvest index (Paull and Chen, 2003). However, citric acid alters sucrose perception (Schifferstein and Fritjers, 1990) and pineapple clones may have sufficient sugars but high citric acid may mask some of the sweetness perception. This masking of sucrose means that higher acid fruit may be perceived as being sour.
Low acid pineapple clones have been available for a number of years though high acid clones are preferred for canning. The availability of cultivars or clones of other economically important fruits differing in acid content, has facilitated comparative studies of organic acid metabolism in apple (Beruter, 2004), peach (Genard et al., 1999 and Moing et al., 1998a), citrus (Sadka et al., 2001) and grape (Diakou et al., 2000 and Terrier et al., 2001). The final organic acid content of fruit is determined by the net balance of acid synthesis, degradation, utilization and compartmentation (Laval-Martin et al., 1977, Ruffner et al., 1984, Muller et al., 1996 and Yamaki, 1984). The enzymes potentially involved in fruit acid metabolism are citrate synthase (CS, EC 4.1.3.7), aconitase (ACO, EC 4.2.1.3) (Sadka et al., 2000a, Sadka et al., 2001 and Luo et al., 2003), phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31), malate dehydrogenase (MDH, EC 1.1.1.37) and malic enzyme (ME, EC 1.1.1.40) (Hirai and Ueno, 1977, Moing et al., 2000 and Diakou et al., 2000). MDH catalyzes the interconversion of malate and oxaloacetate in the cytoplasm. CS catalyzes the acetylation of oxaloacetate using acetyl-CoA to yield citrate that is isomerized by ACO to isocitrate. PEPC condenses phosphoenolpyruvate with bicarbonate to yield oxaloacetate and ME reduces and decarboxylates malate to pyruvate. Although studies had been reported on the compositional changes in some pineapple fruit clones (Kermasha et al., 1987, Bartolome et al., 1995 and Brat et al., 2004), information on fruit acid metabolism is limited. Potassium ion is thought to be involved in organic acid charge balancing (Lang, 1983) and potassium fertilization does increase pineapple titratable acidity (Py et al., 1987 and Spironello et al., 2004), though potassium accumulation in pineapple fruit has not been reported in relation to fruit acidity.
**Ferulic acid**

The hydroxycinnamic acid (HCA) ferulic acid (FA) occurs ester-linked to the primary cell walls (PCWs) of all families of the commelinoid group of monocotyledons. This group contains approximately half of all the monocotyledon families and comprises the following orders recognized by the Angiosperm Phylogeny Group (1998): Arecales, Commelinales, Poales, Zingiberales and some unplaced taxa (Harris; Rudall; Harris; Smith and Harris). However, most of the research done on FA ester-linked to monocotyledon cell walls (CWs) has been done on economically important species of the family Poaceae (Poales) which comprise the grasses and cereals. In contrast to the monocotyledons, FA occurs ester-linked to the PCWs of dicotyledons only in the order Caryophyllales that contains the family Chenopodiaceae that has a number of economically important species, including spinach (*Spinacia oleracea*) and sugar beet (*Beta vulgaris*). FA is ester-linked to polysaccharides in PCWs, but it is linked to different polysaccharides in the PCWs of the Poaceae and Chenopodiaceae. The polysaccharides to which the FA is ester-linked and the exact location of the link were determined by treating isolated PCWs with commercial fungal ‘cellulases’ such as ‘Driselase’ which is from the fungus *Irplex lacteus* and contains a mixture of endo- and exo-glycanases, but lacks hydroxycinnamoyl esterase activity.

Feruloyl oligosaccharides (FOs) were isolated from such enzymic digests and their structures determined. FA is ester-linked to glucuronoarabinoxylans (GAXs), typically the most abundant non-cellulosic polysaccharides in these PCWs (Kato; Ishii; Ishii and Wende). The FA is esterified via its carboxyl group to the C(O)5 hydroxyl of single α-L-Araf residues located on the C(O)3 of Xylp residues in the polysaccharide backbone. In contrast, the two most abundant FOs in enzymic digests of PCWs of the Chenopodiaceae were usually *O-[2-O-E-feruloyl-α-L-Araf-(1→5)-L-Araf* and *O-[6-O-E-
feruloyl-α-D-Galp]-{(1→4)-D-Galp from the PCWs of spinach and sugar beet (Fry; Fry; Ishii; Colquhoun; Ishii and Ralet). The structures of these indicated that the FA is ester-linked to pectic polysaccharides, the most abundant non-cellulosic polysaccharides in these PCWs (Renard and Thibault, 1993). More detailed studies of the polysaccharides in the PCWs of species in families of the order Poales (Smith and Harris, 1999) and of the polysaccharides in the PCWs of pineapple (Ananas comosus, Bromeliaceae) fruit (Smith and Harris, 1995) confirmed the presence of GAXs, similar in structure to those in the PCWs of the Poaceae.

Pathogenesis

Cell wall degrading enzymes produced by Ceratocystis paradoxa

Extracellularly expressed exudates from pineapple fruit infected by Ceratocystis paradoxa were partially purified and the exudate-filtrate preparations were assayed for the presence of three hydrolytic enzymes. The preparations were found to contain cellulolytic and proteolytic enzymes when inoculated on carboxymethylcellulose and casein as substrates, respectively, while pectin-methylesterases and polygalacturonases were detected in the exudate-filtrate when apple pectin and sodium pectate were used as substrates, respectively. The activities and stabilities of these groups of enzymes were found to be optimal at pH 7.0 and at 30 °C. The exudates also exhibited macerating action when potato discs showed complete loss of coherence and maceration after a 24 hr incubation. More total reducing sugars were detected in exudates of infected fruit than those obtained from healthy fruit.
Cellulase

Plant cell walls are a major reservoir of fixed carbon in nature. In recent years there has been considerable interest in the utilization of plant material as a renewable source of fermentable sugars that could be subsequently converted into useful products such as liquid fuels, solvents, chemicals, food, or feed (Clarke, A. 1997; Bothast, and Saha, 1999). Such bioconversion processes are particularly attractive for the elimination of residues and wastes produced by agriculture and forestry. As a result of this interest, a wealth of knowledge on cellulolytic enzymes has accumulated (Béguin and Aubert, 1994; Tomme, et al.,1995; Ohmiya, et al.,1997). The past fifty years have witnessed remarkable progress in (a) isolation of microorganisms producing cellulases (Reese, and Maguire,1971) (b) improving the yield of cellulases by mutation, and protoplast fusion (Brown, et al.,1986), (c) purifying and characterizing the cellulase components (Bhat, K.M. et al., 1989,1990; Christakopoulos, et al., 1994; Wood, and McCrae,1977, Wood, and McCrae, 1977, 1982; 1986) (d) understanding the mechanism of cellulose degradation (Wood, et al.,1988) (e) cloning and expression of cellulase genes (Beguin, and Anbert, 1993; Begnin, et al.,1983; Begnin, et al., 1983, 1988) (f) determining the 3-D structures of cellulase components (Davies,et al., 1993; Davies, and Henrissat,1995, Ducros, et al., 1995), Spezio, et al.,1993) (g) understanding structure-function relationships in cellulases (Claeyssens, and Tomme,1989,Davies, and Henrissat, 1995) and (h) demonstrating the industrial potential of cellulases (Beguin, and Anbert, 1993; Coughlan,1985; Mandels, 1985) Further background information can be found in other recent reviews (Esterbauer, 1991; Kubicek,1992; Ljungdahl, and Eriksson,1985; Ljungdahl, L.G. and Eriksson, K.E. (1985). This review summarizes the cellulase activity of C. paradoxa and other important pathogenic fungi on fruits and vegetables

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Cellulase in C. paradoxa and other pathogenic fungi

The first report on endoglucanase production by Chalara (syn. Thielaviopsis) paradoxa isolated from olive mill waster water disposal pond was carried out recently Lucas et al 2001. However, its role as a SER disease pathogen has not been established yet. Production of cellulolytic enzymes has been reported in other plant pathogenic fungi, such as Macrophomina phaseolina (Wang, and Jones, 1995, ), Phytophthora infestans (Sachslehner, et al., 1998), or Sclerotium rolfsii (39 Bodenmann, et al., 1985). Cellulolytic enzymes should play a role in the penetration of plant cell walls (Bateman, D. F., 1976). The cellulolytic system of C. paradoxa CH32 consists of at least one endoglucanase and one á-glucosidase, which has been characterized recently (Lucas, et al., 2000). Production of endoglucanase activity in liquid cultures takes place during the late trophophase, coincidently with glucose exhaustion. This behavior seems to be in agreement with the general observation that cellulase systems are repressed in the presence of more easily metabolizable carbon sources, for example, glucose (Bisaria, and Mishra, 1989). Under these conditions, endoglucanase formation in various fungi starts only when the repressing carbohydrate glucose is completely metabolized (Ronne, 1995). In this respect, it resembles endoglucanases produced by other fungi such as Aspergillus niger (Okada, 1985) or Aspergillus niveous (Taj-Aldeen, and Alkenany, 1996).

Cellulolytic enzymes are produced by a relatively few fungi and bacteria produce high levels of extracellular cellulase capable of solubilizing crystalline cellulose extensively (Johnson, E.A., et al., 1982; Wood, 1985; Wood, 1991). So far, most of the studies have been on the cellulase system of aerobic fungi Trichoderma viride, Trichoderma reesei, Penicillium pinophilum, Sporotrichum pulverulentum, Fusarium solani, Talaromyces emersonii and Trichoderma koningii (Eriksson, and Wood,1985; Gong, et al., 1979; Eriksson, and Wood, 1985; Gong, et al., 1979;). Only recently, it has
been recognized that the other microorganisms such as thermophilic aerobic fungi (Sporotrichum thermophile, Thermoascus aurantiacus, Chaetomium thermophile, Humicola insolens), mesophilic anaerobic fungi (Neocallimastix frontalis, Piromonas communis, Sphaeromonas communis) mesophilic and thermophilic aerobic bacteria (Cellulomonas sp., Cellvibrio sp., Microbisa ria bispora, and Thermomonospora sp.), mesophilic and thermophilic anaerobic bacteria (Acetivibrio cellulolyticus, Bacteroides cellulosolvens, Bacteroides succinogenes, Ruminococcus albus, Ruminococcus flavefaciens, and Clostridium thermocellum) as well as actinomycetes (Thermomonospora fusca) also produce active cellulases [Ait, et al., 1979; Bartley, et al., 1984; Beguin, and Anbert, 1993; Begnin, et al., 1983; Bhat, et al., 1993; Bhat, and Maheshwari, 1987). Among the above noted microorganisms, the cellulolytic thermophilic microorganisms are of particular interest, because of their ability to produce thermostable cellulases which are generally stable under a variety of severe conditions including highly acidic and alkaline pH as well as temperatures up to 90°C.

Endoglucanases of C. paradoxa have been reported to act preferentially on amorphous substrates (Wood, 1991). However, degradation of crystalline cellulose by the low molecular mass endoglucanase S from Streptomyces sp. LX has also been reported (Li, et al., 1998). Strains of C. paradoxa also produce other extracellular enzymes. Amylase production by C. paradoxa isolated from the pith of the sago palm was reported (Kainoma et al., 1985). More recently, several strains of C. paradoxa isolated from olive mill wastewater disposal ponds were found to produce laccase activity (Robles. et al., 2000). Among them, the strain C. paradoxa CH32 also produced α-glucosidase activity (Lucas, et al., 2000). The endoglucanase enzyme from C. paradoxa CH32 showed optimum activity under acidic pH values and under moderate temperatures of incubation. In this respect it resembles other endoglucanases produced by mesophilic fungi, although
endo glucanases differ markedly in their pH and temperature optima for activity (Schulein, 1998).

**Mechanism of cellulase activity**

Cellulase is an inducible enzyme system (Kubicek, 1992, Ryu, and Mandels, 1980). All microorganisms studied so far have produced the highest level of cellulase when grown on cellulose (Wood, 1985). Cellobiose, lactose and sophorose are also known to facilitate the production of either complete or incomplete cellulase system by few microorganisms (Mandels, and Reese, 1960, Nisizawa, 1971, Ryu, and Mandels, 1980). Synthetic compounds such as palmitate and acetate esters of disaccharides and thiocelllobiose have also been shown to function as inducers of cellulases (Ryu, and Mandels, 1980). However, cellulose was found to be the best carbon source for the production of high levels of cellulase by many microorganisms (Ryu, and Mandels, 1980, Wood, 1985).

The most generally accepted view of induction process is that the low levels of cellulase constitutively produced by the microorganism, first hydrolyses cellulose to soluble sugars (Beguin, and Anbert, 1993, Kubicek, 1992). These sugars are presumably converted into true inducers, which enter the cell and either directly or indirectly influence DNA binding protein and promote cellulase gene expression. It has also been suggested that in case of Trichoderma, the conidial bound cellobiohydrolase hydrolyses the cellulose and releases cellobiose and CBL (cellobiono-tS-1,5-1actone). The cellobiose and CBL are taken up by the mycelia and promote cellulase synthesis (Kubicek, *et al.*, 1988). The extracellular cellulase components of most fungi are generally found to exist as individual entities (Coughlan, 1985). Although many fungi secrete separate cellulase components into
culture medium, it is not yet clearly known how these components interact on the surface of crystalline cellulose and affect the extensive hydrolysis of cellulose.

**Pectinase**

The pectic enzymes were found to cause the maceration and cell killing that is the characteristic of the disease. Pectic enzymes and their products have also been implicated in elicitation of different host defense reactions such as elicitation of phytoalexin (Bruce and West, 1982; Davis *et al.*, 1986). These enzymes were also secreted by post-harvest pathogens viz., *Botrytis cinerea, Sclerotinia fructigena, Fusarium oxysporum, Pyrenochaeta lycopersici, C. lindemuthianum, Colletotrichum gloeosporioides and Alternaria alternata* as part of their strategy for penetrating the plant host cell walls (Scott and Fielding, 1985). They were primarily involved in the degradation of macro-molecules of host cell wall to facilitate their entry (Fogarty and Kelly, 1979). A convincing role of these enzymes in pathogenesis has been established by several workers (Lakshmesha, 2006; Brown and Adikaram, 1982).

Pathogenicity of *C. paradoxa* was found to depend upon the ability to produce cell wall degrading enzymes viz., cellulase rather than pectinase. A detail review of literature is not presented. In addition it also depends upon the stage of ripening of fruits and vegetables during storage. Wherein, there was an increase in the production of endogeneous cellulase and pectinase with an advance in ripening or during storage period in pineapple and other fruits and vegetables (Kertesz, 1951; Padmini Nagaraj, 1987).
Control Measures

Physical control measures

Various physical methods like UV illumination and Radiation was tried on C. paradoxa in vitro. The result indicated prolonged time of UV illumination and high and unpermissible level dosage (>8 kGy) to get desired result of inactivation of C. paradoxa reproductive propugules and young mycelia. Further practical difficulties to obtain uniform illumination of UV or radiation for pineapple fruit warrant restricting the work for in vitro only. The results presented were of academic interest. Important literature review of these treatments is presented in this review. However available literature regarding hot water both in vitro and in vivo is presented.

Ultraviolet illumination

Ultraviolet (UV) illumination is known to damage plant DNA and to affect several physiological processes (Stapleton, 1992). However, a special interest has recently been drawn to the ability of low doses of UV-C light (wavelength of 190-200 nm) to induce disease resistance in a wide range of fruits and vegetables due to induction of phytoalexins (Rodov et al., 1995; Rodov et al., 1992; Droby et al., 1993; Lu et al., 1991) Pathogenesis related (PR) proteins (Portal et al., 1999; El Ghaouth, 1994; Mercier et al., 1993). The temporary effect of UV-C treatments was demonstrated in citrus frufts inoculated with P. digitatum (Droby et al., 1993a).
**Radiation**

Radiation is used for food preservation, the inhibition of sprouting in potatoes and onions, control post harvest diseases (Thomas 1985; Barkai-Golan 1992), delay ripening (Maxie and Abdel-Dader, 1966; Mukherji et al., 1995) and control of insect infestation for quarantine purpose. It may however, adversely affect quality (Barkai-Golan 1992). Radiation sources that have been used include gamma rays (cobalt 60 or caesium 137) and fast electrons (linear accelerators) each have its merits and limitations. Radiation systems are costly but can be easily integrated with other storage and handling methods and are now used mostly in the food packaging industries.

**Radiation approval**

List of commodities approved for radiation by health authorities in various countries has been considerable lengthened. Gamma irradiation up to a dosage of 1 kGy was approved by the Food and Drug Administration of the United States (Barkai-Golan 1992).

**Hot water treatment**

Stem end rot or Black rot of pineapple is controlled by the application of specific fungicides (Liu and Marcano, 1973, Sridhar, 1975 and Cho et al., 1977). However, increasing consumer resistance and the restrictions imposed on the use of these chemicals have created an urgent need for the development of safe and effective alternatives.
Heat treatment technology is a safe and environmentally friendly procedure with increasing acceptability in commercial operations. It is used successfully, to control the incidence of postharvest disease in several commodities (Fallik, 2004). Pre-storage heat treatments to control decay are applied for short periods of time (min), as target pathogens are present in the outer-most layers of host tissue. Water is the preferred medium of application as it is a more efficient medium of heat transfer than air (Lurie, 1998). Pre-storage hot water treatments, methods of hot water immersion and treatment duration have been reviewed by Fallik (2004). The thermal death point of $C.\ paradoxa$ is recorded as 52.5-53 °C (Ames, 1915). Hot water dip treatment at 53 °C followed by TMTD (Thiram) resulted in a limited control of the pathogen in sugarcane propagules (Buergo et al., 1989). However, a hot water dip treatment of 1 min at 52 °C slowed the rate of rot development in litchi (Olesen et al., 2004). Thus, the use of hot water dip treatments as a means of controlling incidence of black rot in Mauritius variety pineapples was investigated.

**Chemical control measures**

A detail account of chemical measure employed to control SER disease of pineapple has been presented. However in the present investigation extensive in vitro and in vivo work was carried out by using GRAS, preservatives and antioxidants which are either constitutive in fruits or inductive due to infection. These chemicals are used for the first time on $C.\ paradoxa$. Hence review of literature in this regard is not available. However appropriate literatures were cited to interpret out results.
Control measures for SER disease of pineapple

Black rot is commercially managed by minimizing bruising of fruit during harvest and handling, by refrigeration and with chemicals. Fruit must be dipped in an appropriate Fungicide within 6–12 h following harvest prior to packing and shipping (Rohrbach Phillips, 1990). Internal-browning symptom development can be reduced by waxing with paraffin polyethylene waxes at wax-to-water ratios of 1: 4–9 (Rohrbach and Apt, 1986). Waxing has been shown to increase internal CO₂ concentrations, thereby lowering O₂ concentrations, which results in reduced polyphenol oxidase (Paull and Rohrbach, 1985). The Penicillium-induced FCR, LP and IFC fruit diseases have been reduced by applications of endosulphan (3.35 kg a.i. /ha in 2338 l water) at forcing and 3 weeks following forcing. Reductions have been significant but only under low to moderate disease levels (Le Grice and Marr, 1970; Rohrbach et al., 1981; Rohrbach and Apt, 1986). Fungicides, such as benomyl, have not been effective unless applied directly into the open heart as the inflorescence emerges (K.G. Rohrbach, unpublished results). Control of typical FCR induced by F. subglutinans has not been demonstrated. Control of fusariosis is most effective by planting disease-free seed material and by controlling insects, particularly the bud moth (Laville, \1980). Hot-water treatment of seed material at 54°C for 90 min with benomyl at 50 g /100 L is effective for disinfestation but will retard growth and kill up to 50% of the plants (Maffia, 1980). Fungicides, such as captan, at 700 g a.i./ ha, starting at differentiation through harvest at 20-day intervals, have given good control of the fruit-rot phase in Brazil (Bolkan et al., 1978). Resistance to fusariosis occurs in Ananas and Pseudoananas (Laville, 1980). Scale can be controlled relatively easily by preharvest applications of an appropriate registered insecticide, taking into consideration last application to harvest residue restrictions.
Both pineapple butt and black rot are caused by the fungus *C. paradoxa*. The severity of the problem in fresh fruit is dependent on the degree of bruising or wounding during harvesting and packing, the level of inoculum on the fruit and the storage temperature during transportation and marketing. Currently, these diseases are controlled by dipping the crown or fruit in a fungicide prior to planting or shipping of the fruit (Cho *et al.*, 1977). Treatment must be done in 12 h or less from the time the crown or fruit is removed from the plant (Rohrbach and Phillips, 1990). Inoculum levels on fruit at harvest vary according to the environmental conditions prior to harvest. The high correlation between moisture (rainfall duration) prior to harvest and disease following harvest has resulted in the name water rot. Storing seed material on the mother plants during dry weather, where there is good air circulation and minimal exposure to inoculum-infested 244 K.G.

Stem end rot is commercially controlled in fresh fruit by minimizing bruising of fruit during harvest and handling and with chemicals. Fruit must be dipped in a fungicide within 6–12 h following harvest prior to packing and shipping. Currently fruit can be dipped in triadimefon. The ‘Queen’ cultivar is generally more susceptible to *C. paradoxa*.

In accordance with the objectives and work carried out in this investigation, review of literature was emphasized on the etiology of disease, biochemical changes in pineapple in relation to ripening and during pathogenesis of *Ceratocystis paradoxa*. Cell wall degrading enzymes a major pathogenic factor of *C. paradoxa* was presented accordingly.