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Sugarcane (*Saccharum spp.*), and Sugarcane Smut (*Ustilago scitaminea* Sydow) the Infection Process, Screening for Resistance, Disease Resistance and Possible Resistance Mechanisms

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Abstract **Review Article**

The interaction between sugarcane and sugarcane smut has been studied for over 130 years, yet we have not fully understand the complete defense mechanisms used by sugarcane plants against the fungus. This article outlines a brief history of sugarcane, sugarcane smut –an overview-, the infection process, some assessment methods for resistance, resistance of sugarcane to smut and there are proposed resistance mechanisms that might be operating at each stage of the colonization of the fungus in the sugarcane plant. The results based on what research has shown, indicate that there is preform and induce resistance operating in the sugarcane-*Ustilago scitaminea* system, both structural and chemical. It is determined that the resistance of sugarcane-to-sugarcane smut is multifactorial and the b pathogen elicits non-specific responses. The 5 step proposed resistance mechanisms involves (a) the role of morphological structure of the bud as it impedes smut spore germination or possibly delay the infection process, (b) the mechanisms used to impede or prevent spore germination on the bud surface, possibly involve diffused substances both preformed and induced. The induced substances being known phytoalexins, (c) the response mechanisms in the sugarcane bud to prevent the establishment of fungal mycelia-hypersensitive response, phytoalexins (phenolics) accumulation, lignification –induced responses, (d) the prevention of mycelia growth in the stalk-Gels and tyloses, glucanases and peroxidases, oxidative burst and savaging, callose deposition, (e) the resistance that might be operating to prevent smut whip formation-antitoxins, hormones, peroxide vessels, oxidative burst and scavenging, callose deposition and possibly papillae. In this overview there is the use *Ustilago scitaminea* to represent sugarcane smut although *Sporisorium scitamineum* is mentioned in some of references.

Keywords: Cultivars, preform resistance, induced resistance, hormones, enzymes, structural, morphological, sugarcane, sugarcane smut, chemical, *Ustilago scitaminea*, *Sporisorium scitamineum*.

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INTRODUCTION

Commercial sugarcane has a chromosome number in the range of 2n=100-125 (Simmonds, 1979). All sugarcane species can interbreed, and the major cultivars are complex hybrids (Vilela, Mariane de Mendonca *et al*., 2017), Sugarcane is known to be a cash crop but it is known to be used as livestock fodder. Sugarcane accounts for over 70% of the sugar produced globally mainly from the cultivar *Saccharum officinarum* L which has the distinction of being the world's largest commercial crop by production quantity.

Among the diseases of great economic importance is sugarcane smut (*Ustilago scitaminea*). Because of the great need to produce sugar without great yield loss it is important to monitor the effect of sugarcane on old and new cultivars. Breeding program are used to produce resistant varieties through methods to assess the cultivar's resistance before it is grown commercially.

Although the interaction between sugarcane and sugarcane smut has been known for over a century we are now beginning to unravel the true nature of resistance of sugarcane to smut. According to research it is determined that the resistance of sugarcane to smut is multifactorial. Different sugarcane mechanisms seem to be employed at different stages of the infectious process.

This article reviews a brief background of sugarcane, a detailed analysis of the smut fungus, the infection process, a short summary of the assessment methods, and a review of resistance mechanisms in sugarcane against the smut fungus. And a five step process of where resistance to sugarcane smut might operating. Despites the genomics, there is still need to assess for the metabolites used by the host to defend against the pathogen. The five step proposed sugarcane resistance is based on known genomics, morphological (structural) features, preform and induced resistance – both structural and chemical and known enzyme and hormone activity.

Sugarcane

Sugarcane is a species of tall, perennial grass of the genus *Saccharum*, tribe Andropogoneae and family Gramineae that is used for sugar production. The mature plants are 5-20 feet tall with stout, jointed, fibrous stalks rich in sucrose (Papini-Terzi *et al*., 2009). Commercial sugarcane has a chromosome number 2n=100-125 (Simmonds, 1979). All sugarcane species can interbreed, and the major commercial cultivars are complex hybrids (Vilela, Mariane de Mendonca *et al*., 2017).

Different species of sugarcane were domesticated. Sugarcane, *Saccharum officinarum* was first grown by the Papuans in New Guinea, another *Saccharum sinense* was first cultivated by the Astronesians in Taiwan and southern China and *Saccharum barberi* was cultivated in India after the introduction of *Saccharum officinarum* (Simmonds, 1979; Daniels and Daniels, 1993, Paterson *et al*., 2012). The sugarcane stems grow into cane stalk which on maturing makes up 70% of the entire plant. A cane stalk when fully mature is composed of more than 10% fiber, more than 12% soluble sugar, 2-3% non- sugar carbohydrates and more than 55% water. However, there is a variety which is high in fiber and low in sucrose (LSU Ag.Center, 2019) which can be used to produce bagasse for fuel and electricity use. Sugarcane is mainly a cash crop but it is also used as life stock fodder (Perez, Rena, 1997).

Sugarcane accounts for over 70% of the sugar produced globally mainly from the cultivar *Saccharum officinarum* and its hybrids (Royal Botanical Gardens, 2012). Sugarcane, *Saccharum officinarum* is the world's largest commercial crop (Rajput *et al*., 2021). In 2020 more than 1.8 billion tons of sugarcane was produced. This makes sugarcane, grown in tropical and sub-tropical regions, the world's largest crop by production quantity (United Nations Food and Agriculture Organization, 2020).

Sugarcane Smut (*Ustilago scitaminea* Sydow)

In addition to disease caused by environmental stress and genetic effects there are at least 85 different parasitic diseases of sugarcane (ISSCT Standing Committee on Sugarcane Diseases, 1974). Among the diseases of great economic important is sugarcane smut which is caused by the basidiomycetous fungus, *Ustilago scitaminea* Sydow. The fungus was first observed in Natal, South Africa in 1877 as Ustilago sacchari (Luthar *et al*., 1940 in Lee-Lovick, 1978). It is now known to occur in all sugarcane producing regions of the world except Fig.1 (Bhuiyan *et al*., 2021).

The severity of the disease at the time of its arrival in a particular country depends upon the acreage of resistant and susceptible cultivars and factors affecting the dissemination of spores and their germination. Pathogenic races and environmental conditions also affect the severity of the disease (Akalach and Touil, 1996; Croft *et al*., 2000). Sugarcane smut is the most serious and widely spread disease of sugarcane and causes significant reduction in cane quantity and quality (Rajput *et al*., 2021). Losses in yield which can vary from 20-70% (Singh and Agnihotri, 1978; Hoy *et al*., 1986) and sugar content are the major problems economically (Bachchav *et al*., 1979, Hoy *et al*., 1986).

Smut disease spreads mainly via different mechanisms (a) wind-blown smut spores are carried in storm and wind currents, (b) disease can be transmitted through diseased propagation material and contaminated farm machinery (Bhuiyan *et al*., 2021), (c) soil borne smut spores can also cause the disease (Comstock, 2000; Croft and Brathwaite, 2006). It is postulated that airborne smut spores is the major cause of smut spores spreading world- wide (Ferreira and Comstock, 1989). The taxonomy of the smuts is based largely on teliospore morphology with spore size, color, ornamentation and shape being especially important (Lee-Lovick, 1978). Mundkur (1939 cited in Lee-Lovick, 1978) studied *Ustilago scitaminea* in detail and subdivided it into *Ustilago scitaminea var sacchari-barberi* and *Ustilago scitaminea var sacchari-officinarum.* His basis for classification was size, color and pattern of the spore wall. Other workers divided sugarcane smuts in Argentina into six groups, one of which approximates Mundkur's var *Saccharum officinarum.* Whether or not a relationship exists between the morphological characteristics of the spores and the pathological strains is uncertain. Gillaspie *et al*., (1983) compared the pathogenecities of six different isolates of sugarcane smuts from different geographical regions and decided each isolate represented a different race of *Ustilago scitaminea*. However, a similar study by French workers failed to show any differences in the germination, virulence or electrophoretic patterns of three enzymes systems between the smut isolates from different parts of the world (Peros and Baudin, 1983). To date there is a new classification of sugarcane smut which is *Sporisorium scitamineum* (Bhuiyan *et al*., 2021).but the old classification *Ustilago scitaminea Sydow* is still widely used.

Sexuality occurs in smut with haploid compatible cells fusing to give a diploid condition

(Alexander and Srinvasan, 1966, Comstock, 2000) and this same heterothallism is evident in isolates from different parts of the world (Peros and Baudin, 1983). The smut spores are spherical, smooth walled or slightly papillate (Sealy, 2020; Commonwealth Mycological Institute Descriptions of Pathogenic fungi and bacteria, NO. 80). Smut spores are light brown to black in color and have a diameter between 5-10 um (Comstock, 2000; Commonwealth Mycological Institute Descriptions of Pathogenic Fungi and Bacteria, NO.80). Smut spores have no dormancy and germinate and germinate readily over a wide range of temperatures (5-40 degrees centigrade) with an optimum between 25-30 degrees Centigrade (Waller, 1969; Sealy, 2020). The optimum spore concentration for smut spore germination is 10^5 spores per ml and the optimum pH for smut spore germination is pH 7.00 (Sealy, 2020). Spore germination can occur at pH 4.00 to pH 8.00 (Sealy, 2020). Bud diffusates, sugarcane debris and leaf washings all stimulate smut spore germination (Waller, 1969; Singh and Agnihotri, 1978). The spores can remain viable for more than three years if stored under dry conditions but readily deteriorate within weeks in humid conditions. Trione (1980) developed a procedure for the culture of the fungus in vitro, starting with explants of the basal pith tissue of freshly cut whips. In vitro, the formation of teliospores was different from that in the sori on the sugarcane whip tissue. The fact that sugarcane extracts were present in the culture medium suggests that as for *Ustilago spp* (Day and Castle, 1982 host substances play a role in the reproductive development of *Ustilago scitaminea*. However, it should be mentioned that Sealy and Carrington (1988) observed the inhibition of smut spore germination in the bud extracts of sugarcane cultivars. This inhibition did not correlate with the field resistant ratings of the sugarcane cultivars but this might be due to the different concentrations of promotery and inhibitory substances in the different cultivars, and the difference in the role of such compounds in the resistance mechanisms of the different cultivars.

The Infection Process

Before infection occurs the smut spores must germinate on the surface of the sugarcane bud (Sealy, 2020). The germination time is 6 hours (Bock, 1964; Waller, 1969; Trione, 1980; Sealy, 2020). Once the germ tube has protruded, this promycelium becomes four celled (Bock, 1964; Waller, 1970). Each promycelial cell is capable of budding off single yeast like sporidia on the bud surface (Sealy, 2020). The sporidia once formed produce infection hyphae on the bud surface (Waller, 1970; Sealy, 2020). Bock (1964) from his own work and others, states that the promycelium produces long, septate hyphae which act as infection threads. Sealy (2020) observed infection observed infection threads, sporidia, and appressoria, all on the surface of sugarcane buds using scanning electron microscopy. The promycelium (germ tube) is capable of direct penetration on the bud surface (Bock, 1964; Sealy, 2020). Dikaryotisation presumably occurring in the host. Dikaryotic infection hyphae can form from paired promycelia following spore germination (Waller, 1969; Sealy, 2020). The fusion between sporidia, promycelia, and resulting in dikaryotisation also occurs on the bud surface (Sealy, 2020). Dikaryotisation occur on the bud surface by infection hyphae (Alexander and Ramakrishnan, 1980), Sealy (2020) also observed dikaryotisation of promycelial strands and a dikaryotic appressorium on the bud surface.

Alexander and Ramakrishnan (1980) found that the fungus entered the bud 6-36 hours following imbibition of water by spores and that it did so by circumventing rather than penetration of the bud scales. Fawcett (1944) observed that infection occurs at the base of the bud, beneath the outer scales. Muthusamy (1974) studied the variation in susceptibility to sugarcane smut in relation to bud sprouting, bud size, bud shape and position of the germ pore. He observed a strong correlation between the smut incidence and bud sprouting in standing canes. According to Muthusamy (1974) the position of the germ pore was subapical in most resistant varieties and apical in susceptible ones. Correlations were also found between smut incidence and the bud size. Cultivars with larger buds were more likely to sprout and be infected by smut. Dormant buds are more resistant than germinating buds (Waller, 1970). Waller (1970) suggested that the increased susceptibility of germinated buds is associated both with the swelling of the bud and an increased of the area within the bud. He thought that the swelling would permit easier access of spores between bud scales, and the increased in area would allow for greater probability of penetration by infection hyphae. Infection hyphae on reaching the meristematic region of the bud is said to undergo a period of latency. The mycelium in the host then spreads intercellularly, feeding through haustoria (Waller, 1970; Singh and Agnihotri, 1978; Alexander and Ramakrisknan, 1980). Lloyd and Pillay (1980) found that the fungus in the cane stalk was largely associated with peripheral vascular bundles especially the xylem. Other workers have reported the fungus presence in the parenchyma adjacent to vascular bundles (Alexander and Ramakrisknan, 1980) and even the phloem (Peros and Chagvarchef, 1984). Bock (1964) noted that the infected bud produces a shoot and the growth of the mycelium of the pathogen keeps pace of the meristematic region. Croft and Brathwaite (2006) found that sugarcane smut grows within the meristematic tissue and induced the formation of flowering structures which it colonizes to produce smut spores. The flowering structure typical of grass arrows are transform into a whip like sorus that grows between the leaf sheaths (Comstock, 2000). However, Glassop *et al*., (2014) demonstrated that none of the flowering genes are expressed during the formation of the sorus (smut whip) and it is now recognized as an elongated internode rather than an inflorescence (Marques *et al*., 2017; Piepenbring *et al*., 2002).

Individual pockets of mycelia remain in each primordium which subsequently develops into a nodal bud (Bock, 1964). Mycelia are absent (Bock, 1964) or least abundant (Lloyd and Pillay, 1980; Fereol, 1984) in internode regions. The condition is only systemic because all nodal buds contain individual pockets of mycelium. Singh and Agnihotri (1978) stated that the fungus can be isolated from infected apical and lateral bud meristems of canes showing the initial symptoms of the disease. After infection the major symptom of the disease is the production of a smut whip. Other unusual symptoms such as mass proliferation of side shoots, sori development in flowering panicles, parts of the inflorescence turned vegetative, and the initiation of vegetative shoots from panicles with sori have been documented (Bhuiyan *et al*., 2021). The smut whip is a black, whip-like structure which grows from the central core of the meristematic apex to the maximum length of 90 centimeters.

Immediately before whip production there is an increase in the activity of the apical meristem and the rapid accumulation of mycelium at its periphery (Waller, 1969). The growth pattern of the apical meristem changes and it becomes intercalary in function, acting as the basal meristem of the smut whip (Sealy, 1988). Trione (1980) observed that the fungus grew rapidly in the developing whip relative to other sugarcane tissue. In the modified apex, the vegetative hyphae changed physiologically and cytologically into the reproductive phase that yield a large number of spores. Trione (1980) found that the vegetative hyphae in the sori located in the surface layers of the whip were mononucleate and irregular in shape and length. Hyphae in the sori on the outer portion of the whip were different from those in other parts of the whip (Trione, 1980). These hyphal cells aggregated, then enlarged and release their nuclei into a gelatinous matrix following hydrolysis of their cell walls (Trione, 1980). Cytoplasm and cell wall then gradually form around each nucleus to form spores about 8 um in diameter. As spores matured the outer walls became pigmented and the gelatinous matrix holding the spores together dried out (Trione, 1980). Spore dispersal occurred at this stage once the outer peridium had fragmented (Trione, 1980).

The early stages of whip emergence are variable and depend on the rate at which young leaves surrounding the whip unfurl and the rate of whip growth. Bock (1964) showed that temperature affects the rate of whip production and that 30^0 C was the optimum. Smut whips grow for about 12 weeks and during that time the diseased canes increase in height by a maximum of 2.0 M. The faster growth rate of diseased canes often results in old smut whips being above the canopy, therefore facilitating spore dispersal. A smut whip can produce about 10^9 spores per day and a total of 10^{11} spores during its growing period (Waller, 1969). In dry conditions the spores are rapidly shed from the emerging whips which have been exposed for over two days. In wet conditions spore dispersal is hindered and most spores form a hard cake on the whip.

The systemic nature of the disease means that any shoot primordial produced from an infected meristem will also be diseased. This leads to the systemic infection of the secondary tillers produced by plants which were infected during their first stages of growth. Such plants can produce a succession of smut whips for lengthy periods during the growth of the crop (Waller, 1969). Vander der Plank (1963) found that several successive generations of a parasite can occur during the growth of a crop and this leads to the multiplication of the disease. Waller (1969) observed that the smut incidence increased from 0-100% over a 22 month period and that there is a latent period of six months in the field. The proportion of smutted plants increases markedly with the successive ratoon crops-secondary crops from existing root stocks (Antoine, 1969).

Both rain fed and surface irrigation conditions increased the diseased incidence (Bock, 1964; Waller, 1969). The deposition of spores in the field is variable (Waller, 1969). In crops where the canopy is dense few spores reach the soil beneath. The downwind side of the crop shows the highest deposition of the spores. Freshly deposited spores showed a viability of 80% (Waller, 1969). Spores are deposited on all exposed surfaces of the cane. Those deposited on the upper leaf surface are thought to be washed into leaf axils by rain and the majority become lodged around the nodal bud of the cane.

Infected plants are generally stunted with thin stalks and with narrow, stiff leaves often at an acute angle. In exceptional cases smut galls are seen on young leaves with an off-white membranous covering which on rupturing exposes smut spores (Singh and Agnihotri, 1978). Despite the different measures used to control smut such as hot water treatment (Gupta, 1979) and fungicides (Bhuiyan *et al*., 2015), the most effective control measures are planting resistant varieties, planting healthy setts, removing whips as they appear and ploughing out diseased ratoons (Bock, 1964; Waller, 1969; Singh and Agnihotri, 1978; Bhuiyan *et al*., 2021).

Assessment of Host Resistance

Inoculation, both artificial and natural, is the major method used for the assessment of host resistance. The various methods of inoculation summarized after Lee-Lovick (1978), Sealy (2020) and others are:

(a) Spores are applied at the ends of cuttings.
(b) Spores dusted on buds at planting.
(c) Spores mixed in soil before planting.
(d) Soaking cuttings in spore suspension (Bachchav *et al*., 1979; Whittle, 1982).
(e) Spores applied to young buds on standing canes.
(f) Spores introduced into wounds at base of shoots or around buds or foots.

(g) Application of spores to cane flowers and the resultant infected seeds grown.
(h) Inoculation of uprights (Benda and Koike, 1985)
(I) Dip minus bud scale inoculation (Rampersad and Brathwaite, 1985).
(J) Inoculation of standing canes in a humid chamber (Sealy, 2020)
(K) Inoculation of buds on setts and assess for spore germination (Sealy, 2020)

The most commonly used technique for the inoculation of buds is dipping in an aqueous suspension of spores. Various modifications in time of dipping and spore concentrations are used (Byther and Steiner, 1974; Bachchav *et al*., 1979; Dean, 1982; Whittle, 1982). For natural inoculation in the field, it is widely accepted that ratooned canes give a truer picture of the resistance of various cultivars to smut (Walker, D.I.T, Personal communication.

Resistance of Sugarcane to Smut (Disease Resistance)

There are mechanisms that are thought to be used by plants to defend against pathogens. The defensive mechanisms can be divided into two major categories: (a) Preform Resistance Factors, (b) Induced Resistance Factors. Both of these barriers can be divided further into structural and chemical factors. As their names suggest one refers to factors existing prior to the arrival of the pathogen (Preform), while the other refers to factors developing after the arrival of the pathogen (Induced).

Preform structural features includes the plant's cuticles, leaf waxes or bud waxes, stomatal type, plant hairs, and secretary glands (Sealy, 1988). Preform chemical substances includes flavonoids, alkaloids, phenolics and sulphur compounds- all known as secondary metabolites (Sealy, 1988; Osbourn, 1996). Extrude materials are also secondary metabolites that reach the plant surface and defend against the pathogen (Sealy and Carrington, 1988; Sealy. 1988). Microbial antagonism on the plant surface can be considered a preform factor because any organisms which inhabit plant surfaces and are inhibitory to pathogen development will enhance the resistance of the plant to the pathogen.

Induced resistance is brought about as a response of the host plant to the presence of the pathogen. The host can be sensitized by the presence of fungal spores, germ tube penetration, mycelial development, or fungal establishment (Hare, 1966; Bailey and Mansfield, 1982). Induced resistance can be elicited by fungal chemical components (Sealy, 1988). Induced structural features include cell wall modifications such as callose deposition, accumulation of calcium and silicon, suberization, melanization, lignification and the impregnation of cell walls with phenolic compounds (Sealy, 1988). Induced chemical resistance factors are comprised of two important mechanisms, namely, phytoalexins and hypersensitivity. Phytoalexins are a group of secondary metabolites such as phenolics, flavanoids, isoflavonoids, stilbenes, and alkaloids produced to inhibit pathogenesis in plants. Hypersensitivity is a necrotic event induced in host plants to inhibit invading pathogens. Hypersensitivity is widely thought of as an induced secondary response (Sealy, 1988). Phytoalexins are also associated with hypersensitivity responses in some plant-pathogen systems (Sealy, 1988). It should be noted that plants and pathogens evolved together and during this co-evolutionary process pathogens developed systems which enable them to parasitize plants, whereas plants develop sophisticated mechanisms to defend themselves.

With regard to the resistance of sugarcane to sugarcane smut it is determined that the resistance of sugarcane to sugarcane smut is multifactorial (Sealy, 1988; Sealy and Carrington, 1988; Dean, 1982; Bhuiyan *et al*., 2021). This phenomenon is not new to plants since Misaghi (1982) reported that disease resistance in plants is multifactorial. There are two barriers to infection in sugarcane to sugarcane smut (Dean, 1982) and characterized as a pre-infection barrier and a post infection barrier (Lloyd and Pillay, 1980). They suggest that the pre-infection barrier is associated with the bud scales which provide both chemical and physical resistance to the entry of the promycelium. The post infection barrier occurs after the fungus enters the host and is probably more chemical than physical (Lloyd and Pillay, 1980). Bhuiyan *et al*., (2021) reported that there is an external and internal barrier to infection whereas Sealy (1988; 2020) suggest that there is preform and induced resistance in sugarcane to the smut fungus. Sealy suggested that both resistance mechanisms can be both physical (structural) and chemical. Bud morphology provides physical resistance to the entry of the infection hyphae. Singh and Budhraja (1964) suggested that bud scales are barriers against infection probably by delaying or preventing infection hyphae reaching the meristematic cells. They observed that the application of viable spores over a bud after removing the scales, followed by incubation and planting the inoculated seed-piece resulted in 100% infection in the susceptible cultivars of sugarcane. Appezzato-da-Gloria (1995) presented early evidence that resistant cultivars exhibited preform structural features against smut infection. The researched reported that the resistant cultivarSP70-1143 possessed a higher number of bud scales, with lignified cells, phenolic compounds accumulated in the epidermis and a higher number of trichomes (hairs) were present as compared to the susceptible cultivar –NA56-79.

Waller (1970) found that there is a correlation between a number of bud characters and resistance to smut, namely, the presence of a flange, bud groove, type of germination, bud size, time to burst and the growth rate. Muthusamy (1974) studied the variation in susceptibility to smut in relation to bud sprouting, bud size, bud shape, position of the germ pore and the

incidence of the stalk borer (Chilo indicus) A strong correlation was observed between smut incidence and bud sprouting in standing canes, He observed that the sprouting of buds in two varieties was due to the attack of the stalk borer. However, the high percentage of sprouting and the presence of the stalk borer did not always predispose cultivars to smut. According to Muthusamy (1974) the position of the germ pore was subapical in most resistant varieties and apical in susceptible ones. Correlations were also found between smut incidence and bud size. Cultivars with large buds were more likely to sprout and be infected by smut.

Plant hairs may be involved in the resistance of pathogens by host plants by secretion of toxic substances (Hafix, 1952; Misaghi, 1982; Weinhold and Hancock, 1980), There is no known relationship between the presence of bud hairs on the surface of sugarcane cultivars and resistance to smut, However, bud hairs may be important chemically as secretary structures since there are secretary glands at their apex (Sealy, 2020). To a lesser extent hairs could prevent the direct contact of spores with the bud surface depending on their closeness (Bhuiyan *et al.,* 2021). Whether the response to the presence of the pathogen by the hairs is spontaneous or constitutive in terms of chemical reaction is unknown. But an interesting observation (Sealy, 2020) is the presence of a secretary structure in a socket at the top of a hair. This secretary structure probably falls to the bud surface releasing its contents and the upright structure remains showing a circular hollow (Sealy, 2020).

Studies have suggested that preform chemical factors leaching out of the bud tissue into the moist film on the bud surface may inhibit smut spore germination and represents one line of the plant's defense (Lloyd and Naidoo, 1983; Lloyd and Pillay, 1980; Rampersad and Brathwaite, 1985; Sealy and Carrington, 1988; Bhuiyan *et al*., 2021). Glycosidic substances in the bud scales were found to be associated with smut resistance in some sugarcane cultivars (Lloyd and Naidoo, 1983; Fontaniella *et al*., 2002; Millanes *et al*., 2008). Bhuiyan *et al*., (2021) mentioned glycosidic flavonoid compounds in external resistance suggesting that such compounds are preformed and diffuse to the bud surface. Biochemically, glycosidic compounds within sugarcane tissues can inhibit smut spore germination (Millanes *et al*., 2008). Such compounds have been identified as glycoside flavonoid compounds (Bhuiyan *et al*., 2021). Bud characteristics therefore represent preform structural factors and the diffused substances represent preform chemical factors. Bud hairs may represent preform structural as well as preform chemical factors.

Physiological and biochemical changes were observed during the early stages of the infection process when sugarcane buds were inoculated via injection of a spore suspension (Su *et al*., 2016a). They found that the physiological and biochemical changes were related to resistance. The method did not address the structural and chemical resistance produced by bud scales (Aitken *et al*., 2013; Bhuiyan *et al*., 2013). However, the idea of physiological and biochemical changes occurring within the plant supports the concept that there is induced resistance-both structural and chemical.

Internal resistance is thought to consist of a cascade of defense mechanisms induced by the pathogen -induced resistance- (Bhuiyan *et al*., 2021). This clearly suggests that there is induced resistance in the bud, stalk, and in defense of whip formation. Studies have shown that the response of sugarcane to *Ustilago scitaminea Sydow* is complex and involves many aspects of biological activities (Bhuiyan *et al*., 2021). Pathway enrichment analysis revealed that in the sugarcane-sugarcane smut interaction differentially expressed genes are involved, plant hormone signal transduction, phenylalanine metabolism, peroxisome, flavonoid biosynthesis, phenylpropanoid biosynthesis and ribosome and other resistance associated metabolic pathways-all induced response-chemical and structural (Bhuiyan *et al*., 2021). An upregulation of secondary metabolites associated with the flavonoid and phenylpropanoid pathway occurred during the early stages of the pathogen infection in smut resistant cultivars (Marques *et al*., 2018; McNeil *et al*., 2018). The activation of the phenylpropanoid pathway is an active defense response in plants that leads to the production of chemicals with antimicrobial activities-anthrocyanidin, phenolic compounds such as coumarins, stilbenes, neo-lignins, flavonoids and phenylpropanoid conjugates – known phytoalexin products. (Meides *et al*., 2014: McNeil *et al*., 2018; Oliveria *et al*., 2016).

In addition to the synthesis of antimicrobial compounds the phenylpropanoid pathway is an important metabolic pathway leading to the synthesis of lignin (Bhuiyan *et al*., 2021). Lignin is essential in the defense against pathogens because the induced lignification of the cell wall can prevent the diffusion of toxins and enzymes of the pathogen into the host and therefore presents an undegradeable mechanical barrier to most pathogens (Kawasaki *et al*., 2006; Koutaniemi *et al*., 2007). Lignification occurs in many plants in response to infectious microorganisms and in some instances to wounding (Vance *et al*., 1980; Ride, 1983). Ride (1978) suggested 5 ways in which lignification might hinder fungal growth:

1. Lignin might increase resistance to mechanical penetration by fungi
2. Lignin deposition at the infection site might increase resistance to fungal enzyme attack and degradation.
3. Lignification of cell walls might restrict the flow of nutrients to the pathogen and toxins to the host.
4. Low molecular weight phenolic precursors of lignin and free radicals produced as a result of polymerization, might inactivate fungal

components of pathogenesis (enzymes, membranes, toxins and elicitors).

5. Hyphal tips might become lignified and growth stopped due to the loss of plasticity.

Sue *et al*., (2016b) showed that protein involved in the lignin biosynthetic pathway were induced by *Ustilago scitaminea* in cultivars which were resistant to smut. They findings were confirmed by other workers (Barnabas *et al*., 2016; Bedre *et al*., 2019; Huang *et al*., 2018; McNeil *et al*., 2018; Que *et al*., 2014a; Schaker *et al*., 2016). The studies support the idea that lignification is an induced structural and chemical response of sugarcane to the smut fungus. In resistant sugarcane cultivars early lignification, oxidative burst, and the up regulation of chitinases, flavonoids, and classic disease resistant genes prevented smut whip formation (Bhuiyan *et al*., 2021). Marques *et al*., (2018) reported that lignin and phenolic compounds accumulated during the early stages of the sugarcane smut infection. They also reported that later in the infection process there is a characteristic protective layer with lignin, cellulose and arabinpxylan in the cell walls.

Lignins are phenolic polymers of hydroxycinnamyl alcohols. They are made up of three different components, coniferyl, sinapyl and p-coumaryl alcohol units. These combine to form many different types of lignins. Phenolic compounds have been reported to have antimicrobial activity (Kuc, 1976; Friend, 1977; Bailey and Mansfield, 1982; Goodman *et al*., 1986).

Melanization is another cell wall modification that appears to be relevant for disease resistance. Melanin is a complex aggregate of quinoid pigment and enzyme systems in a protein matrix. In plants the enzymes polyphenol oxidase and peroxidase oxidizes colorless dihydroxyphenols to colored orthoquinones. Some hydroxyphenols may conjugate with each other or hydroxyl groups of glucose to form tannins. These colored tannins and quinones condense to form melanins (Bell, 1981). Melanin formation is highest in some resistant plants suggesting that melanins or their precursors are of importance in plant resistance to pathogens (Bell, 1981). Lazarovitz and Higgins (1976) found that the concentrations of dihydroxyphenols were related to cultivar resistance. Caffeine related compounds have been related to varietal resistance in water melons to *Alternaria cucumerina* (Chopra *et al*., 1974), and chili peppers to *Colletotrichum* anthracnose disease (Bhullar *et al*., 1972). Hecker *et al*., (1975) reported that 3, 4-dihydroxyphenyalanine (DOPA) in cultivars resistant to *Cercospora* leaf spot disease was consistently higher in resistant cultivars than susceptible cultivars for the entire growing season. They observed that both DOPA and resistance declined with age. In the sugarcane-sugarcane smut system Sealy and Carrington (1988) found that the unknown inhibitory compound that inhibited smut spore germination in sugarcane bud extracts seem to declined with age. The 7 month old cultivars showed greater inhibition than 9 month old cultivars and the 9 month old cultivars showed more inhibition than the 12 month old cultivars. In the Cercospora leaf spot disease system Cartwright and his colleagues (1980) reported an enhancement of phytoalexin production by diclorocyclopropane. This implies a complementary nature in defense mechanisms. Not all examples where chemicals which enhance melanin production gave consistent correlations with disease resistance (Bell, 1981), but this might be due to the complementary effect of other defense mechanisms.

Leath and Rowell (1969) reported that callose, a B-(1-3) glucan increased in content in thickened wall of corn mesophyll cells in response to invasion by *Puccinia graminis*. A thickened cell wall and callose deposition occurred in healthy cells surrounding necrotic lesions caused by *Botrytis cinerea* in both susceptible and resistant corn cultivars (Garcia-Arenal and Sagasta, 1977). However, Lazarovits and Higgins (1976) found larger deposits of callose and greater wall thickening in resistant compared to susceptible tomatoes to races of *Cladosporium fulvum*. Batcho and Audan (1980) also reported changes in callose and other host cell wall components of Silene dioica infected with *Ustilago violacea*. It is clearly indicated that callose deposition might be a defense mechanism in plants. Similarly, calcium and silicon may also assist in fungal impediment by strengthening the cell wall (Sherwood and Vance, 1980). Sugarcane cells of the sorus deposited callose at *Ustilago scitaminea* sites of penetration and surrounding its intracellular hyphae, indicati9ng a possible role of callose as an induced structural defense (Marques *et al*., 2017). They also observed callose in the sieve plate and throughout the xylem of the sorus. This callose deposition seems to be a response to preventing smut whip formation. Su *et al*., (2016) reported that a calcium signaling pathway may be repressed during resistance to *Ustilago scitaminea* infection of sugarcane. Su *et al*., (2016) concluded that calcium signaling and other pathways which were repressed by *Ustilago scitaminea* might not be important in smut resistant of sugarcane.

Papillae are formed at the outer surface of a cell wall in response to penetrating hyphae, appressoria or wounding (Aist, 1976; Ride 1978). They are composed of callose, lignin, cellulose and inorganic ions like silicon, calcium, phosphorus and potassium (Aist, 1976; Sherwood and Vance, 1980; Kunoh *et al*., 1986). Papillae are also known as callosities, lignitubers or appositional wall thickenings. Vance and Sherwood (1976) implicated papillae formation as a mechanism of epidermal resistance to *Phalaris arundincea* and in graminaceous plants to direct penetration by nonpathogenic fungi. In 1980, Sherwood and Vance presented information supportive of the idea that papillae are biosynthesized. Different species of graminaceous plants were inoculated with *Stemphylium botryosum*. Of 781 epidermal sites where the fungus initiated penetration 779 developed papillae in the epidermal cell

walls and penetration was unsuccessful. At the other two sites the wall failed to thicken and penetration occurred. When leaves were floated on cycloheximide before inoculation, *S. botryosum* penetrated 767 of the 771 sites examined. There was no wall thickening. Cycloheximide, an inhibitor of protein synthesis, inhibited papillae formation. Whether or not papillae are involved in the sugarcane- sugarcane smut system is unknown. However, since most of the components necessary for papillae formation present in the sugarcane system it is quite possible that there are papillae. Research is necessary to determine whether or not papillae are involved.

Another defense mechanism employed by plants against pathogens is the establishment of the hypersensitive reaction within the infective tissue that is often regulated by reactive oxygen species (ROSs) and nitric oxide with the induction of program cell death (Zaninotto *et al*., 2006). A hypersensitive response in the classical sense, of plants to pathogens stipulates that host cell necrosis precedes the restriction of fungal growth (Maclean *et al*., 1974). An oxidative burst is produced in response to infection with fungal pathogens and causes a direct toxic effect to the invading pathogen along with localized injures to the plant cell membrane (Heller and Tudzynski, 2011). This process delays or impairs fungal colonization and is also part of a signaling cascade activating hypersensitivity response, cell wall modification, the synthesis of antimicrobial compounds (phytoalexins), and gene expression changes (Smirnoff and Arnaud, 2018; Thordal-Christensen *et al*., 1997).

Peters *et al*., (2017) demonstrated that during *Ustilago scitaminea* and sugarcane interaction an increase in H_2O_2 concentration produced by the plant reduced fungal colonization in a smut resistant genotype. Peters and Colleagues (2017) were able to relate each fungal development stage to the variation of the response of the sugarcane plant. Early symptoms were observed and reported to become macroscopically visible in resistant and susceptible genotypes which included chlorosis and small necrotic spots (McNeil *et al*., 2018: Peters *et al*., 2017). This suggests some sort of hypersensitive response.

Muller and Borger (1940) proposed that plant produce substances in their defense against infection. The defensive substances were called “phytoalexins”. Since then phytoalexins have been extensively studied and reviewed (Kuc, 1976; Cruickshank, 1980; Keen, 1981; Bailey and Mansfield, 1982; Goodman *et al*., 1986). Phytoalexins comprise a diverse group of compounds (Deverall, 1982). Bazzalo *et al*., (1985) demonstrated an increase in phenolic compounds in sunflower stems inoculated with *Sclerotia sclerotiorum* and these compounds when extracted, inhibited the mycelial growth of the fungus. Extracts from resistant cultivars were more inhibitory. Isochlorogenic acid had the strongest effect of all the components found in the extracts. Inoculation of broad bean roots with *Fusarium oxysporum* stimulated accumulation of the isoflavonoid pterocarpan phytoalexin (Ibrahim *et al*., 1982). Its antifungal activity was demonstrated on the mycelial growth and conidial germination but a good correlation was not obtained between root resistance of several broad bean cultivars and the concentration of phytoalexin. This suggest that resistance is multifactorial and other mechanisms are involved along with phytoalexin accumulation.

In grasses, phytoalexins also appear to be involved in resistance. Lim *et al*., (1968) reported that phytoalexins may be important in resistance of corn to *Helminthosporium turcium.* In a similar system, Conture *et al*., (1971) using cultivars which differed in monogenic resistance, suggested resistance expressed 48-72 h after inoculation was partly due to the presence of preformed hydroxamates, whereas resistance occurring after that time was dependent on the accumulation of phytoalexin. Lloyd and Pillay (1980) found that diffusates from intact sugarcane buds held in *Ustilago scitaminea* spore suspensions for 18 h contained higher concentrations of four flavonoid glycosides than buds held in distilled water. The flavonoid concentration was highest in the resistant cultivar, lowest in the susceptible cultivar and intermediate in the moderately resistant cultivar. These relative concentrations remained unchanged despite the absolute increase in flavonoid level following exposure to the pathogen. These results imply phytoalexin behavior and suggest that *Ustilago scitaminea* elicits a response for phytoalexin accumulation in the sugarcane-sugarcane smut system.

Using RNA sequencing studies to examine the initial response to smut infection indicated that *Ustilago scitaminea* infection elicits a strong non-specific response in sugarcane (Marques *et al*., 2018; McNeil *et al*., 2018; Peters *et al*., 2017; Que *et al*., 2014a; Su *et al*., 2016b). Like other inducible responses phytoalexin accumulation is associated with elicitors and suppressors. Darvill and Albersheim (1984) characterized elicitors as being biotic or abiotic. They classified abiotic elicitors as substances or conditions not found in living tissue and biotic elicitors as derived from the pathogen or host. Elicitors have also been characterized as being specific or non-specific. Elicitors which have differential induction activity in various plant cultivars depending on the different resistance genotype are termed specific elicitors whereas non-specific elicitors are those which do not have such differential activity (Yoshikawa, 1983). The elicitor hypothesis implies that glucans, glycoproteins, and other cell wall products of plant pathogens are responsible for the accumulation of phytoalexins. This is also true for other induced responses by the plant in defense of the pathogen. Suppressors are thought to be released by the pathogenic fungi to make the host plant susceptible (Oku *et al*., 1977; Doke *et al*., 1980a). The suppressors that a

pathogen uses to overcome host derived reactive oxygen species (ROSs) are regarded as either enzymatic, including superoxide dismutases and peroxidases such as glutathione peroxidase and catalase (Ghelfi *et al*., 2011), or non-enzymatic, consisting of the synthesis of small soluble molecules that are oxidized by ROSs such as Glutathione and antioxidant compounds, namely, phenolics, flavanoids, carotenoids, glycosidic compounds, ergothioneine and ascorbic acid (Sanchez, 2017). Extracellular oxidoreductases, such as laccases, glutathione-S-transferases and raffinose, which are known to be associated with scavenging free radicals after the attack by the pathogen (Arnstadf *et al*., 2016; Mauch and Dudler, 1993) have been noted to be induced after infection of sugarcane by Ustilago scitaminea (Peters *et al*., 2017; Schaker *et al*., 2016; 2017).

Lipid peroxidation is a known biochemical marker of oxidative stress (Gratao *et al*., 2005). Lamb and Dixon (1977) proposed that lipid peroxidation is a key process for membrane alterations in plants. In many instances this response is efficient against biotrophic pathogens, like sugarcane smut, that depend on living cells for survival (Koeck *et al*., 2011). Enzymes that are activated in diseased plants have been regarded as having a role in disease resistance. Such enzymes as peroxidases, polyphenol oxidases, and phenylanaline ammonia lyase have been implicated (Bell, 1981; Glazener, 1982). Since these enzymes are involved in the synthesis of melanins, phenolics and lignin (Maule and Ride, 1976; Vance and Sherwood, 1976; Bell, 1981), it may well be acting through the synthesis of such compounds that the resistance factors of enzymes are related. Song *et al*., (2013) determined that ascorbate peroxidases and thioredoxin-dependent peroxidase were upregulated in a smut resistant genotype at day 3 after infection and suggested that the enzymes could remove excessive ROSs -which might aid the infectious process of the fungus-and protect the sugarcane plant from smut infection. A case for oxidative scavenging. The genes for the enzymes S-adenosylmethionine synthetase and aminocyclopropane-1-carboxylic acid are over expressed during *Ustilago scitaminea* infection (Schaker *et al*., 2017). Bhuiyan *et al*., (2021) suggested that the upregulation of S-adenosylmethionine synthetase might have promoted the accumulation of polyamines in response to the invading pathogen. de Armas *et al*., (2007) showed that the sensitivity or resistance to sugarcane smut was correlated with changes in free phenolic compounds as well as phenylanaline ammonia lyase and peroxidases activity in host leaves induced by an elicitor from *Ustilago scitaminea* mycelium. The results, based on known the literature, suggest that induced structural features such as melanins and lignins and/or their precursors might be involved in the resistance of sugarcane to the smut fungus. The changes of free phenolics through oxidation would suggest the induction of other compounds- flavonoids (phytoalexins) in the resistance of sugarcane to the smut fungus. Peters *et al*., (2017) showed that in a smut resistant genotype the *Ustilago scitaminea* hyphal growth was delayed and visibly accumulated in peroxide vesicles. This suggests that peroxide is a possible preform substance in the internal resistance of sugarcane to the smut fungus. It would be interesting to determine if this phenomenon occurs in the sugarcane stalk.

There is early evidence to suggest that plant hormones play a signaling role in the resistant response in plants (Pegg, 1976; Elstner, 1983; Goodman *et al*., 1986). The progression of *Ustilago scitaminea Sydow* infection is known to be accompanied by a distinctive transcriptional change in different plant hormone genes (Bhuiyan *et al*., 2021). Plant hormones such as abscisic acid, ethylene, gibberellic acid, and gibberellin and auxin activation were induced in the response of sugarcane to *Ustilago scitaminea* attack (Bhuiyan *et al*., 2021; Que *et al*., 2014a; Maximova *et al*., 2006; Su *et al*., 2016b). The role of these hormones may represent induced structural or chemical responses or both. It might be that they induced chemical responses such as methylation of nucleic acids, proteins, lipids, polysaccharides and polyamines (Bhuiyan *et al*., 2021).

Proposed Resistance Mechanisms-Biochemical, Structural (Physical)

Resistance is the ability of an organism to exclude or overcome, completely or in some degree hinder, the effect of the pathogen (Agrios, 1988). Disease resistance in plants is manifested through limited systems, reflecting the inability of the pathogen to grow or spread and multiply and is often associated with a hypersensitive reaction in which the pathogen remains confined to certain necrotic legions near the sight of infection (Van Loon, 1997). Resistance can be preformed or induced. Both involve physical (structural) factors as well as chemical factors. Induced resistance in plants to pathogens was first recognized early in the 1900 (Ray, 1901; Beauverie, 1901). Induced resistance in plants was defined as an increased expression of natural defense mechanisms of plants against different pathogens (Edreva, 2004). Induced resistance is divided into categories, namely, Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) (Edreva, 2004; Choudhary *et al*., 2007). SAR is triggered by the accumulation of Salicylic acid and the ISR is dependent on signal transduction pathways activated by the Jasmonic and ethylene (Bhuiyan *et al*., 2021; Yan *et al*., 2002). Both categories of induced resistance can be activated by biological inhibition of the pathogen whereas ISR is a resistance mechanism that does not depend on the direct factors such as fungi and their metabolites (Walters *et al*., 2013). SAR can be systemic or localized in its killing or inhibition of the invading pathogen, but instead on increasing the physical (structural) and chemical barrier of the host plant (Edreva, 2004; Choudhary *et al*., 2007). There tends to be a maintenance balance in the activation of both SAR and ISR responses. As one goes up it inhibits the effect of the other (Traw and Bagelson, 2003). There is cascade

of molecular and biochemical events that underlines the expression of SAR.

Since the resistance of sugarcane to sugarcane smut is suggested to be multifactorial it is expected that different resistance mechanisms are involved in combating the fungus' initial infection, its establishment, and to inhibit or kill the fungus. It is therefore expected that some resistance mechanism(s) will be involved at each stage in the colonization of the sugarcane plant by fungus as it leads to smut whip formation by the pathogen. The flow diagram -Figure 1 outlines 5 possible levels at which resistance mechanisms may be employed to overcome the progression of the fungus. Induced resistance is mentioned without differentiation to either the SAR or ISR.

The first level of resistance is based on the correlation of the preform structural and morphological characteristics of the sugarcane bud with disease resistance in the sugarcane-sugarcane smut system. Waller (1970) found that there is a correlation between bud characters such as the presence of a flange, bud groove, type of germination and growth rate. Muthusamy (1974) found that the position of the germ pore was related to smut resistance as well as bud size. Singh and Budhraja (1964) reported that bud scales are barriers to infection of the smut fungus probably by delaying or preventing infection hyphae reaching the meristematic region of the plant. Glycosidic substances in the bud scales were found to be associated with smut resistance in some cultivars (Fontaniella *et al*., 2002; Lloyd and Naidoo, 1983; Millanes *et al*., 2008). Sealy (2020) observed hairs on the bud surface with secretary glands and suggested that the hairs are important for resistance, both on a preformed structural basis and a preform chemical basis due to their secretary glands. The idea that plant hairs might be involved in disease resistance was reported earlier in different host- pathogen systems (Hafiz, 1952; Misaghi, 1982). While epidermal hairs may contribute to resistance, they seem to do so more by virtue of the chemicals they contain rather than their physical presence (Hafiz, 1952; Misaghi, 1982; Sealy, 2020). Toxic substances secreted by plant hairs may be involved in resistance (Hafiz, 1952; Weinhold and Hancock, 1980; Misaghi, 1982). It is therefore noted that there is strong preform physical (structural) resistance to sugarcane smut on the bud surface as well as some preform chemical resistance in the form of bud hairs and their secretary glands, Structural features alone might not protect plants from invasion by pathogens, but can provide a delay which may or may not have an effect on the outcome of the disease in some host pathogen systems. The delay they provide the plant may allow time for a more effective defense response (Royle, 1976; Misaghi, 1982; Conti *et al*., 1982).

The second level of the flow diagram (Fig 1) represents the mechanisms involved in the prevention of smut spore germination. It is evident from research that in the sugarcane-*Ustilago scitaminea* system there is resistance to spore germination on the bud surface but it appeared to be more chemical than structural. Sealy and Carrington (1988) and Sealy (2020) reported a good correlation between resistance of sugar cane to smut and the Mean Percentage Germination of smut spores on the bud surface and the resistance ratings of sugarcane cultivars. They showed that there is clearly a trend of inhibition of spore germination with increasing resistance to smut of the 10 cultivars used. Sealy and Carrington (1988) suggested that although structural features are involved in resistance (Waller, 1970), preform antifungal substances present in bud diffuses to the surface and inhibit spore germination. There seem no grounds for the involvement of phytoalexins in the case of Sealy and Carrington (1988) findings since in the same smut-sugarcane system phytoalexin accumulation took longer than 6 hours (Lloyd and Pillay, 1980). Induced substances (phytoalexins) are known to reach the bud surface and inhibit smut spore germination after 6 hours of imbibition (Lloyd and Pillay, 1980). It is now known that some such substances that reach the bud surface are at least 4 glycoside flavonoid compounds and other glycosidic substances (Lloyd and Pillay, 1980; Bhuiyan *et al*., 2021). It is clear from levels 1 and 2 in the flow diagram which showed the effects of bud morphology, diffused preform substances and phytoalexin accumulation that the bud surface is a major form of resistance in the sugarcane-*Ustilago scitaminea* system.

The third level at which resistance mechanisms may come into play is in the preventing the fungus entering and establishing itself within the sugar cane bud (Fig 1). The expression of genes for programmed cell death (Zaninotto *et al*., 2006) and the observation of chlorosis and necrosis (McNiel *et al*., 2018; Peters *et al*., 2017) suggests that a hypersensitive reaction may be operating in sugarcane buds against the fungal pathogen (smut). All induced by an oxidative burst in the sugarcane-*Ustilago scitaminea* system. Hypersensitive reactions associated with phytoalexin accumulation (Van der Plank, 1975). The activation of phenylpropanoid pathway suggests that phytoalexins are being produced in response to the fungal infection by sugarcane smut as well as the formation of lignins. The activation of the enzymes peroxidases (ascorbic peroxidases, theordoxin - dependent peroxidase), phenyloxidases and phenylalanine lyase suggests lignification, melanization, and phenol formation. Hence, induced resistance structures such as lignins are involved at this level for the resistance of sugarcane to the smut fungus (Marques *et al*., 2018). Phenols, known phytoalexins are involved through the activation of the enzyme S-adenosylmethionine synthetase. Enzymes involved in lignin biosynthesis are reported (Su *et al*., 2016b). Lignins and melanines are considered cell wall modifications. Lipid peroxidation confirms such in the sugarcane-Ustilago scitaminea system. Whether there are preform structural features within the bud that

protects against infective mycelia is uncertain but can be determined through histological studies. However, it is recognized that induced structural features are operating at this level. Lignification leading to lignin formation is an associated response in the sugarcane to the smut fungus in the bud scales and along the surface (Marques *et al*., 2018).

Level 4 in the flow diagram (Fig 1) relates to the prevention of mycelia growth in the stalk, Lloyd and Pillay (1980) reported that the fungus in the cane stalk was largely associated with peripheral vascular bundles especially the xylem. Other workers have reported the fungus presence in the parenchyma adjacent to vascular bundles (Alexander and Ramakrisknan, 1980), and even in the phloem (Peros and Chagvarchef, 1984). There is no mention of preformed structural resistance which may be operating. Gels and tyloses, pectins, hemicelluloses, and other carbohydrate material associated with the vascular system, are thought to be associated with the resistance of plants to vascular pathogens. Despite evidence to suggest their importance in resistance their roles are not defined (Bell, 1981). Gels are known to coat walls and fill the lumina of infected vessels and they occur in numerous plant species infected with fungi (Bell, 1981). There are two proposed theories for the origin of gels;

(a) Gels arise from the perforated plates, and walls and pit membranes by a distensible process of the primary wall and middle lamella constituents. Such constituents probably include pectinaceous materials, hemicellulose, and other carbohydrates (Bell, 1981).
(b) Drawing attention to the excessive development of golgi apparatus and other secretary organelles in the paravascular parenchyma, Moreau *et al*., (1977) suggested that new materials, carbohydrates included, may be synthesized and secreted to form gels.

Although the mycelium of *Ustilago scitaminea* is observed in the conducting vessels of infected sugarcane plants (Lloyd and Pillay, 1980; Alexander and Ramakrisknan, 1980; Peros and Chagvarchef, 1984), there is no mention of the involvement of gels or tyloses. This is an area that needs to be investigated in the sugarcane-sugarcane smut system as there is reason to believe that gels may be involve in resistance to sugarcane smut in the stalk. Preform chemical resistance may involve the phenolics present in the stalk. Also oxidative burst and scavenging may be an associated resistance response (Peters *et al*., 2017; Schaker *et al*., 2016; 2017). In a similar manner where callose deposition was observed in the sieve plate and throughout the xylem of the sorus (Marques *et al*., 2017), it would be interesting to know if the same response occurs in the sugarcane stalk in response to the fungal infection. The involvement of glucanases and peroxidases in the internal resistance of sugarcane to *Ustilago scitaminea* (Bhuiyan *et al*., 2021) would suggest that in the sugarcane stalk glucanases break down fungal glucans in fungal (smut) cell walls exposing the cytoplasmic contents which is then oxidized by peroxidases. This involvement of glucanases and peroxidases may impede further fungal development. Hence an induced chemical response.

The final stage at which resistance to sugarcane smut infection is operating is in the prevention of smut whip formation (Fig 1). Hormone signaling might be most effective in the resistance to the development of the smut whip. The hormones ethylene, absisic acid and gibberellic acid are produced in sugar cane infected with smut. These hormones might be operating to prevent the development of the smut whip through the induction of chemical antitoxins and other induced resistance mechanisms both structural and chemical. Toxin binding proteins isolated from resistant and susceptible clones of sugar cane are believed to be important in resistance of sugarcane to *Helminthosporium sacchar*i. The fungus produces a toxin, helminthosporide, which binds to the plasma membrane of susceptible cultivars but not to resistant cultivars (Strobel, 1982). Strobel (1982) suggested that susceptible plants have a toxin binding protein and the toxin protein interaction leads to infection. Analogous to the sugarcane-*Ustilago scitaminea* interaction with the accumulation of polyamines (Bhuiyan *et al*., 2021) in susceptible cultivars one can argue that polyamines serve as a substrate which helps to promote infection. However, any compounds (antitoxins) that inhibit the production of polyamines or neutralizes them would enhance the resistance of sugarcane to the smut fungus. This might be occurring to prevent the formation of smut whips in the sugarcane-*Ustilago scitaminea* system. Callose deposition at *Ustilago scitaminea* sites of penetration and surrounding its intracellular hyphae (Marques *et al*., 2017) suggests that callose deposition may be a defense mechanism against whip production. Additionally, in the prevention of whip formation fungal mycelium might be engulfed in peroxide vesicles (Peters *et al*., 2017). Oxidative burst and scavenging might also be involved (Peters *et al*., 2017; Schaker *et al*., 2016; 2017). The up regulation of chitinases, flavonoids, and classic disease resistant genes prevented whip formation (Bhuiyan *et al*., 2021). Papillae which are known to contain callose, lignin, cellulose and ions such as calcium (Aist, 1976; Sherwood and Vance, 1980; Kunoh *et al*., 1976) might be a resistance mechanism involved since papillae are formed at the outer surface of the cell wall in response to penetrating hyphae, appressoria, or wounding (Aist, 1976; Ride, 1978). The possible involvement of papillae in the sugarcane-*Ustilago scitaminea* interaction needs to be investigated since callose, lignin, cellulose and ions such as calcium appear to be responses associated with the sugarcane-sugarcane smut system.

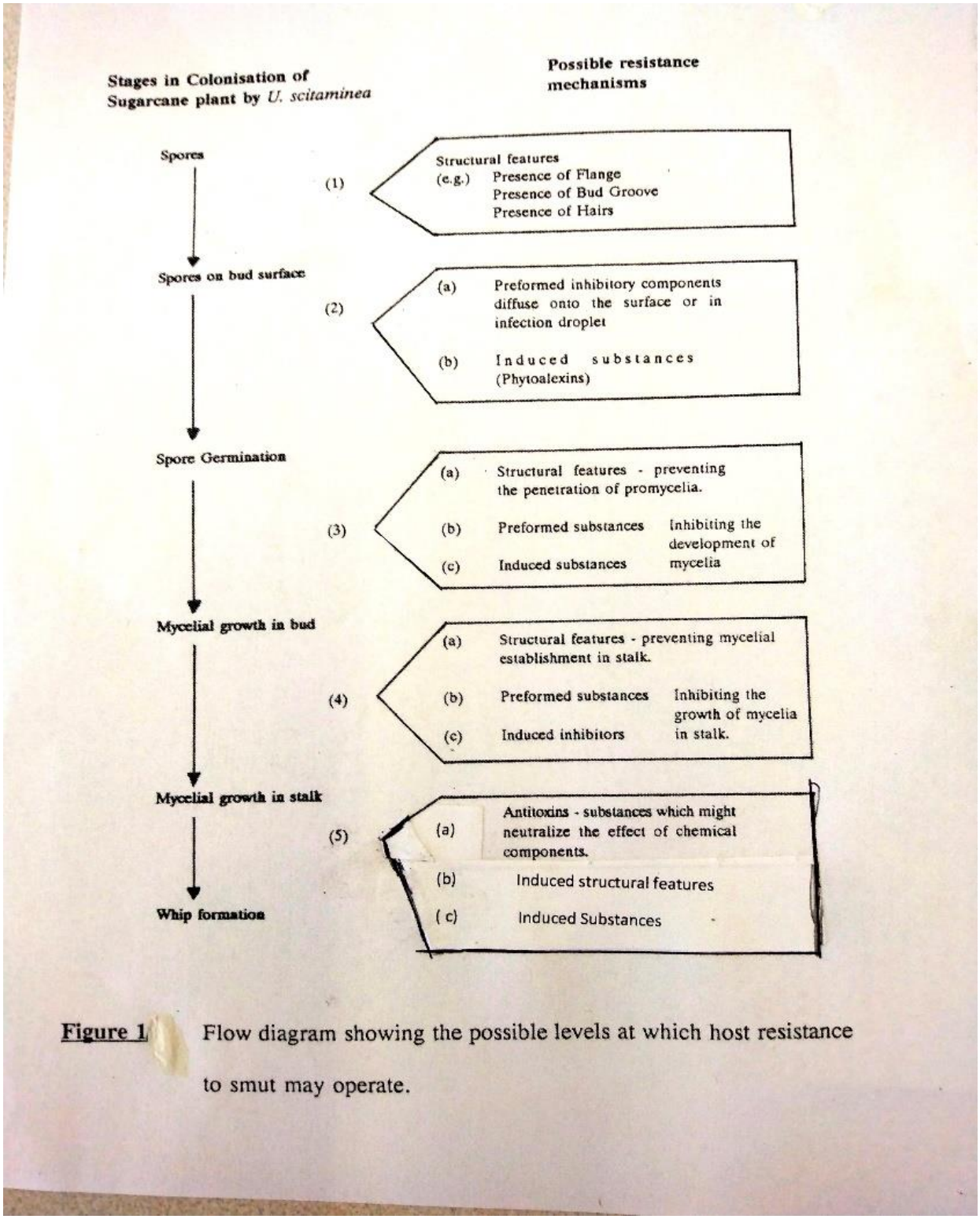

Figure 1 Flow diagram showing the possible levels at which host resistance to smut may operate.

Figure 1

CONCLUSION

In the flow diagram structural features relate to the structural and physical components that are thought to involve in resistance. These structural features can be preformed or induced. Substances mentioned in the flow diagram (Fig 1) are deemed to be all chemical and biochemical compounds that are involved in resistance, hormones and enzymes included. According to the flow diagram (Fig 1) it is safe to conclude that structural features are involved at all levels of resistance except level 2. This is so because the presence of hairs with secretary glands would suggest that resistance through the presence of hairs is probably chemical. With the exception of levels 1 and 2 structural features are thought to be induced through the formation of lignins. Preformed substances mentioned are probably glycosidic

compounds and phenolics. Flavonoids are thought to be induced-phytoalexins. It is clear that at level 5 antitoxins, induced substances and induced structural features might be involved in resistance.

It is clear from the evidence provided that there is a defense employed by sugarcane cultivars against the smut fungus at every stage of the colonization of the fungus in the sugarcane plant. Although there are proposed resistance in the flow diagram (Fig 1) it seems logical from what is known in gene expression, biochemical pathways activated, and the biochemical processes initiated by the enzymes and hormones discussed. There is need for a thorough chemical and biochemical analysis of the plant tissue at each stage of infection process to validate the proposed resistance mechanisms that have been developed. Getting to know what chemicals/biochemicals are involved in each stage of resistance would not only be of value to academia but also breeding programs. It can now be concluded that the resistance of sugarcane to sugarcane smut is multifactorial. It is clear that morphological features of the sugarcane bud are important in the resistance to sugarcane smut; there is hypersensitivity; phytoalexins, preformed substances; enzymes and hormones in the induction of resistance mechanisms and there is engulfing of fungal mycelium in peroxide vesicles. Oxidative burst and scavenging is also involved along with induced structural features-lignins and callose deposition.

Plants contain substances which promote pathogenic development and those that inhibit. Whether or not, the pathogen is inhibited-by spore germination, mycelial development or fungal establishment- depends of the balance of these preform substances in the plant (Hare, 1966). This is clearly the situation in the sugarcane-*Ustilago scitaminea* system. Flor (1955) proposed that for every gene for infection by the pathogen there is a corresponding gene for resistance by the host. This appears also to be the case in the sugarcane- *Ustilago scitaminea Sydow* system.

The resistance of sugarcane to *Ustilago scitaminea Sydow* infection can be considered to be structural, biochemical, chemical, and physiological involving both preformed and induced resistance.

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