A Summary of Recent Research on the Superelongation Disease of Cassava

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SUMMARY

Recent research on pathogen taxonomy, sexual reproduction, host range and physiology are summarized along with investigations into the nature of superelongation disease resistance. The previously unreported sexual stage of *Sphaceloma manihoticola*, causal agent of superelongation disease of cassava, is presented as an *Elsinoë* species (Loculoascomycetes, Myriangiales). Cross-inoculation studies, symptomatology, colony, characteristics, and morphological comparisons among isolates, type specimens, and published descriptions support proposed synonymy of the *Elsinoë* from cassava with *E. jatrophae* and *E. brasiliensis* under the same *Elsinoë brasiliensis*. *Sphaceloma manihoticola* is retained, and *S. krugii* is combined with *S. poinsettiae* under the latter name. *In vitro* production of gibberellin A_4 by the pathogen was demonstrated through combined gas chromatography-mass spectrometry of purified culture extracts. Field resistant and field susceptible cultivars were treated with different concentrations of gibberellin A_4 and inoculated with the pathogen. No difference in response to the hormone was detected between resistant and susceptible cultivars when evaluated for change in length and susceptibility. All cultivars showed an increase in internode length at 10^3 µg/µl hormone concentration, and all showed increased level of disease over control when inoculation followed hormone application. Regardless of field susceptibility, stem tissue of all cultivars tested develop resistance to the pathogen after about 8 days. Hormone production appears to confer an advantage to the pathogen by inducing growth and increasing the amount of susceptible juvenile tissue. Disruption of stem cuticle significantly increases level of disease as measured by number of lesions, percent of susceptible tissue diseased, and number of diseased internodes. Inoculations of selected cultivars of diverse origins and morphology with isolates of diverse origins in all combinations yielded variable, but significant cultivar x isolate interactions suggestive of pathogenic specialization. It is hypothesized that specific host-isolate incompatibility is only part of a complex of resistance mechanisms in field resistant cultivar.

The superelongation disease of cassava, first reported in the Tolima Valley of Colombia in 1972 (3), has since been reported throughout wide areas of South and Central America and the Caribbean. The CIAT cassava pathology section has conducted research on disease etiology, physiology, and epidemiology, and pathogen taxonomy and physiology, much of it in cooperation with Cornell University. This paper summarizes most important results of these studies. Detailed data supporting the findings here have or will be published elsewhere (8, 9, 10, 11).

I. Pathogen Taxonomy and Sexual Reproduction

The genus *Sphaceloma* de Bary (Melanconiales) currently composes more than 50 species, the majority in tropical and subtropical regions. The pathogen attacks flowers, fruits, leaves, and stems, causing characteristic scab lesions as well as necrotic leaf spots. Conidia are small, unicellular and hyaline, formed in a more or less acervulus-like structure or, more commonly, on continuous fertile layers of densely packed phialidic conidiophores. Under certain conditions, some species may form a larger, 0-2 septate, pigmented, thick-walled, spindle-shaped spore. This form, originally described from *Sphaceloma fawcetti* Jenkins (5) has been referred to as the "fawcetti" conidium (7) and has been implicated in long-distance wind dissemination of the pathogen (21).

The genus *Elsinoë* Racib. (Loculoascomycetes, Myriangiales) contains over 40 species and has been shown to be the perfect state of all but one species of *Sphaceloma* for which the sexual stage is known. Host symptoms are identical to those caused by *Sphaceloma* spp. Bitunicate asci, found solitary in locules, usually contain eight hyaline or slightly pigmented ascospores which are commonly 3-septate, often with a longitudinal septum in one or more internal cells.

In 1950 Bitancourt and Jenkins (2) described a species of *Sphaceloma*, *S. manihoticola* on cassava (*Manihot esculenta* Crantz, Euphorbiaceae). Their description and decision to consider it a new species was based entirely on symptomatology and host position within the Euphorbiaceae, since no spores or other fungal reproductive structures were visible in their specimens. They also observed symptoms which they attributed to the same pathogen on *M. glaziovii* Muell. Arg., but made no mention of internode elongation in their description of symptoms. Bitancourt and Jenkins (2) stated clearly that they considered the new species as provisional, pending later opportunity to examine fresh specimens containing reproductive structures.

Symptoms of superelongation disease include necrotic leaf spots, hypertrophic leaf-vein, petiole and stem cankers, characteristic of scab disease caused by *Sphaceloma* (4). In addition, severely affected plants show marked elongation of the internodes, from which the disease name is derived. The causal agent was identified as a species of *Sphaceloma* by Krausz (6) who decided that it should be considered *S. manihoticola*, even though it was impossible to determine if it was the same species described by Bitancourt and Jenkins. Prior to this study, no *Elsinoë* had been reported from *Manihot* spp.

Since 1942 several species of *Sphaceloma* and *Elsinoë* have been described as pathogens on weedy and ornamental plants related to cassava in Central and South America. *Euphorbia brasiliensis*, *Eu. heterophylla* L., *Eu. hypericifolia* L. and *Eu. prunifolia* L. are all weeds susceptible to *Sphaceloma*-*Elsinoë* spp. and common in cassava growing areas. Scab-susceptible *Eu. pulcherrima* Willd. (the "poinsettia") is a common ornamental shrub. *Jatropha curcas* L. as well as *J. aconitifolia* Muell. var. *papaya* Arbelaez are trees often found in hedgerows between fields or as ornamentals in cassava growing areas.

Experimental

Isolates of Sphaceloma spp. were obtained from various Euphorbiaceous hosts in Latin America. These were cultured as described elsewhere (9) and comparisons made of colony, conidiophore, and conidial morphology. Cross inoculations with all the Sphaceloma-Elsinoë species and hosts under consideration were conducted and conidial dimensions from each host compared. The morphology of an Elsinoë spp. found on lesions of SED-affected cassava was studied, and the pathogenicity of single-ascospore isolates on cassava and other Euphorbiaceous hosts tested.

Findings

Colony morphology of the different species of Sphaceloma was so variable as to be of limited usefulness as a criterion for distinguishing species. Conidial dimensions of isolates from several hosts considered to be hosts of distinct Sphaceloma-Elsinoë species were not found to differ significantly. No other reliable means of separating these species could be found. Conidial dimensions of isolates from cassava, Jatropha spp., Euphorbia brasiliensis and Manihot carthaginensis were all found not to differ. Isolates from poinsettia (E. pulcherrima), E. heterophylla, and E. prunifolia, though significantly larger than the former group, did not differ significantly among themselves. The latter group also were found to produce fawcetti conidia, absent in the former group. Cross-inoculations with all possible host-pathogen combinations followed the same pattern as conidial dimensions.

Ascoma were observed on hypertrophic tissue of cassava leaves, petioles, and stems of plants collected during the rainy seasons when rains were regular. These structures were present on material from Mexico (Tabasco), Costa Rica, the Dominican Republic and Colombia (Departments of Casanare, Cauca, Magdalena, Meta, and Vichada). Extensive examination of material from the other host species showing scab symptoms yielded no sexual structures.

Ascoma on cassava are pulvinate (occasionally applanate) and convoluted to smooth, solitary or coalescing, 20-130 μm in diameter. They originate subepidermally and are composed of a hyaline pseudoparenchyma with a distinctly pigmented epithelium, giving the structure a dark appearance when viewed from above. Asci (usually with eight ascospores) are bitunicate and globose (13-22 μm in diameter) and occur singly in locules with poorly developed walls. Mature ascospores (11-14 x 3-7 μm) are hyaline, have transverse septa at which they show slight constriction, and commonly have a longitudinal septum in one or more of the internal cells. One end of the spore is typically somewhat broader than the other. Germination is by production of conidia or direct, with one or more germ tubes produced from each cell, near the septa. Fragmentation of ascospores was occasionally observed, particularly with dried specimens.

Based on comparisons of published descriptions and extensive examination of herbarium type specimens, and the results of the morphology and cross-inoculation studies, Elsinoë antidesmae, E. heveae, and E. venezuelensis are considered to be distinct species and should be retained. However, the differences among E. brasiliensis, E. jatrophae, and the Elsinoë from cassava are not considered sufficiently great to warrant their continued treatment as separate species, and should all be considered to be E. brasiliensis. Sphaceloma manihoticola is, therefore, the correct name for the imperfect state of E. brasiliensis as well as the causal agent of superelongation disease. Similarly, S. krugii does not differ sufficiently from S. poinsettiae to be considered distinct, and is combined with the latter under the name S. poinsettiae.

The host range of E. brasiliensis is considerably expanded and includes Manihot spp., Euphorbia brasiliensis (a cosmopolitan weed), Jatropha curcas and J. aconitifolia var. papaya (ornamental trees). This has important implications for cassava in other parts of the world. It is likely that should S. manihoticola be introduced into Asia or Africa, weedy Euphorbiaceous hosts will prevent its eradication. Similarly, as has been the case with the CIAT station, complete eradication of the pathogen appears impossible. Quarantine programs in areas free of SED should be aware that other Euphorbiaceous hosts notably the attractive ornamental Jatropha spp. may carry cassava pathogens. Finally, considering that there is a common sexual stage, in addition to the wide host range, it is likely that a high degree of pathogenic variability is present in most populations of S. manihoticola.

II. Pathogen Production of Gibberellin A_4

Krausz (6) in early work on the disease unsuccessfully attempted to isolate a fungus-produced plant growth regulator responsible for the internode elongation. Plant hormones are known to be involved in many plant diseases. The classic case is that of bakanae disease of rice caused by Gibberella fujikuroi (Saw.) Wr. from which the plant hormone gibberellin (GA) was first isolated. The most obvious symptom of this disease is internode elongation of juvenile rice plants not unlike that observed in superelongation disease. Given this similarity in symptoms, we felt that a GA-like compound produced by the fungus may be responsible for the hypertrophic symptoms. This portion of the study was undertaken to determine if the pathogen is capable of producing a GA in vitro that reproduces secondary symptoms in the absence of the pathogen. Finding that the case, subsequent studies were conducted to determine if field resistance or susceptibility of cultivars were correlated to sensitivity to the hormone.

Experimental

Isolates of S. manihoticola were grown in liquid medium for 2-3 weeks. Culture filtrate was purified by acid-base partitioning followed by silica gel column chromatography. Fractions from the chromatography were passed through combined gas chromatography-mass spectrometry and compounds identified based on the resulting mass spectra. Biological activity was determined using lettuce seedling hypocotyl bioassay and treatments of young cassava plants with purified hormone.

Young plants of cassava cultivars known to differ in their levels of field susceptibility to SED were treated with increasing concentrations of gibberellin A4. After 7-10 days, depending on experimental particulars, lengths of newly emerged internodes of treated and control plants were measured and plants then inoculated with conidial suspensions of the pathogen. After incubation, plants were evaluated for percentage of stem area diseased and length of diseased area. Data were analyzed using analyses of variance and covariance.

Findings

Results of purification and analyses of fungal extracts demonstrated unequivocally that S. manihoticola produces gibberellin A4 in vitro (11). Application of the purified hormone to cassava plants reproduced the stem elongation and leaf deformation symptoms. The only other fungus known to produce gibberellins is

Gibberella fujikuroi, an unrelated pathogen of rice. All nine cultivars tested responded to applications of GA4 by increased internode length. Significant increases over control were detectable at concentrations of $1x10^3$ µg/µl test solution. At each concentration and over all concentrations the amount and percent susceptible area diseased on field susceptible (FS) cultivars and field resistant (FR) cultivars differed significantly. No relationship between minimum [GA4] required to elicit significant elongation response and varietal susceptibility was detected. While the rates of elongation as functions of increasing [GA4] differed significantly among cultivars there was no difference between FS and FR cultivars when the data were analyzed using analysis of covariance with [GA4] as the covariate and field susceptibility as a class variable.

All cultivars showed increasing amounts of disease with increasing hormone concentration. This suggests that GA4 production may confer an advantage to the pathogen by increasing the amount of susceptible juvenile tissue. As in the case of internode length, rate of change of the amount of diseased area did not vary with cultivar field susceptibility. In some cultivars, high [GA4] not only increased the total amount of disease on a plant, but also increased the percent of susceptible tissue involved in lesions, or tissue susceptibility, with important implications for secondary cycles and epidemic development in the field.

III. Some Characteristics of Resistance

In almost all national and international programs a key approach to improving cassava yields is breeding improved varieties. Because cassava is vegetatively propagated from farmer-produced "seed," long cycle (up to 24 months) and grown with few inputs, a fundamental goal of improvement programs must be varietal stability. The discovery that the sexual stage of the pathogen is common, together with a wide host range, argues that the potential exists for tremendous variability within pathogen populations. Whether within this variability are found high levels of pathogenic specialization becomes of critical concern to breeding programs, since most evaluations for SED disease resistance are carried out at only one site.

Experimental

Several experiments were conducted to test the influence of removing stem cuticle of young cassava plants on subsequent level of disease. Immediately prior to inoculation, stems of plants of cultivars ranging from highly susceptible to highly resistant were subjected to gentle abrasion by a water-soaked cotton swab. This treatment has been found to remove stem culture with minimal damage to underlying tissue. Alternatively, one half of a stem was treated to remove the cuticle while one half was left with cuticle intact. In all experiments an equal number of treated and untreated plants were used. Stem cuticle thickness of young tissue from plants growing in a heavy SED region (Carimagua Station, Meta, Colombia) was measured and level of SED on the cultivars taken at the time of tissue collection.

Cross inoculation studies were conducted using 30 isolates of diverse geographical origin and over 60 cassava cultivars. Space limitations permitted only five to six cultivars to be inoculated with 10-11 isolates in all possible combinations at any one time. Level of disease was determined after 10 days, and was measured as the number of internodes involved (recorded as continuous variable) and the percent of affected stem area directly involved with lesions.

Data from the inoculations were analyzed using a two-way analysis of variance with the primary interest being significant cultivar x isolate interactions. Cultivar x isolate mean tables were inspected for suspicious interactions when significant or near significant F-ratios were calculated for cultivar-isolate interactions. Cultivars and isolates involved in relatively strong interactions were included for repeat experiments to test the stability of the interactions.

Findings

For all cultivars, regardless of known field susceptibility, disruption of stem cuticle significantly increased the amount of disease on the treated stems compared to the untreated stems. However, susceptible cultivars always remained more susceptible than resistant cultivars. Thus stem cuticle is not a primary determinant of resistance. No correlation was found between stem cuticle thickness and level of field resistance. Stem tissue was found to become resistant with age. Stem tissues of all cultivars, regardless of their level of resistance (or susceptibility), became completely resistant after about 10 days. This resistance is unaffected by cuticle removal, and its mechanism is unknown. Removal of cuticle increased the length of diseased stem tissue, thus it is believed that cuticle acts as a defensive barrier over susceptible juvenile tissue until the age-related mechanism develops. Cuticle probably acts simply by preventing the inoculum from adhering to the stem.

Two-way analysis of variance of the results of the inoculations, evaluated as number of infected internodes and percent susceptible area diseased, revealed significant cultivar x isolate interactions for most of the inoculations using the isolates and cultivars selected from the initial group by their performance in the preliminary experiments. Inspection of the results showed several isolates that were commonly involved in the interactions, while others showed no specific interaction with any cultivars used in these experiments. Likewise, some cultivars showed no evidence for specific interaction with the isolates used, while others did.

In light of the significant cultivar interactions, it is not appropriate to consider grand means as reflections of differences in virulence or resistance for the isolates and cultivars. Several isolates consistently had the lowest mean disease levels over all cultivars in different experiments. However, examinations of the mean tables revealed that this was usually because one or two cultivars behaved as though they were differentially resistant to these isolates, lowering the overall isolate mean. Other cultivars were as susceptible to these "less virulent" isolates as they were to the "more virulent" isolates. Thus, while there was some trend indicative of differences in virulence it was not strong.

While high levels of physiological resistance to all isolates was encountered in stem tissue of some cultivars, none were found to be "immune" or to have a hypersensitive-like response. The leaves of all cultivars showed rather high susceptibility under the experimental conditions. Cultivar x isolate interactions typically were such that the cultivar appeared differentially resistant rather than differentially susceptible. Similarly, interactions were rarely "complete." That is, two isolates causing significantly different amounts of disease on one cultivar tended not to reverse order of virulence on another cultivar. Rather, they would cause equal amounts of disease. Another interesting result was that some cultivars that have a high level of apparently stable field resistance showed significant interactions with some isolates and showed levels of physiological susceptibility comparable to those cultivars very susceptible in the field. Thus, factors other than physiological host-pathogen compatibility appear to be involved in the development of superelongation disease epidemics in the field.

It is important to consider in a technique such as that used here to detect host-isolate interactions that one is simply evaluating one, and the first, exposure of the plant to the pathogen. In the field SED is multicyclic, and the ability of the cultivar to limit subsequent generations may be equally, or more, important that the susceptibility at one, or the first, encounter. Any glasshouse evaluation of resistance must include means of evaluating this. Additional parameters of evaluation should include incubation and latency periods, final lesion size, sporulation rate, and conidia size.

To evaluate importance of pathogenic specialization phenomenon within the context of host species and cassava breeding programs, it is necessary to determine whether or not the interactions observed are real. Several independent lines of evidence suggest that, although weak and subject to variation, there is specific adaptation of resistance in some cultivars to virulence factors found in the pathogen population. First, in all inoculations the relative response of a cultivar with known high field susceptibility to the same isolates remained rather constant. There were only two isolates showing significant differences in their relative virulence on this cultivar over different inoculations using the standard technique. These isolates do not enter into evaluations of interactions. Second, statistically significant interactions were repeatable. In general, most cultivars used in the detailed experiments showed interation with some isolates and these were consistent with earlier observations. This is to be expected since most of the cultivars and isolates were selected out of the preliminary inoculations because they appeared to show specific interactions. It is significant that those cultivars which uniformly showed initial very high susceptibility or very low susceptibility with no interactions remained stable in subsequent inoculations over the same as well as other isolates.

Summary of the data in the form of a cultivar x isolate table is not presented here. Presentation of the data in such a form could be misleading and possibly be interpreted as providing evidence for "races." We feel that the relationship between E. brasiliensis and cassava is such that to define "races" and "differentials" would impose wholly arbitrary and simplistic limits on a dynamic and fluid system.

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