Research

Infection Biology of *Crinipellis perniciosa* on Vegetative Flushes of Cacao

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ABSTRACT

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Infection biology of *Crinipellis perniciosa* on vegetative flushes of cacao (*Theobroma cacao*) was studied by fluorescent microscopy. Basidiospores germinated only if they landed on wet tissue or were suspended in water and sprayed onto the plant parts; those that landed on dry surfaces were no longer viable. Interruption of the period of wetness during the prepenetration phase resulted in irreversible termination of the infection process. Growth of germ tubes on the surface of young flushes was strongly oriented toward natural infection sites (stomata) and wounds. On hardened flushes, growth of germ tubes was random. Germ tubes penetrated through stomata on any of the organs of young developing flushes. Penetration into the basal cells of fallen or collapsed multicellular trichomes on fully expanded but unhardened flushes also occurred. Substomatal vesicles were commonly observed in unhardened tissues 12 hr after inoculation. Infection sites were not equally susceptible; in some instances, intercellular hyphae grew freely throughout the infected tissue, whereas in others, mycelial growth stopped shortly after penetration. The failure to colonize host tissue after penetration was associated with a change in the fluorescence of host cells at and around the infection site. Penetration through stomata was also observed on hardened flushes, but substomatal vesicles or intercellular hyphae did not develop.

Additional keywords: cocoa, disease resistance, epidemiology, germ tube tropism, witches'-broom

Vegetative flushes in cacao (*Theobroma cacao* L.) may be the most abundant infection sites for the witches'-broom pathogen. Detailed knowledge of the infection process of *Crinipellis perniciosa* (Stahel) Singer on flushes is essential to clarify the epidemiology of the disease and to improve and develop standard inoculation methods for evaluating resistance of cacao to witches'-broom disease. However, several aspects of the infection process are not well understood.

Factors affecting germination of basidiospores have been studied only in vitro. Sparsely distributed basidiospores collected on dry glass surfaces immediately lost their viability, whereas basidiospores accumulated in high numbers on glass surfaces survived for 15-20 hr (22). Basidiospores in buffered solutions or on agar germinated optimally at pH 6 and 25 C (13). The requirements for germination of basidiospores on plant surfaces and, therefore, optimal conditions for infection of inoculated plants are uncertain.

The mode of penetration has received considerable attention. Germ tubes that entered stomata on leaves and pods and colonized the epicarp of young pods were first observed by Stahel (22). Suarez (23) and Aragundi (1) failed to substantiate Stahel's observations. Cronshaw and Evans (7) reported that *C. perniciosa* penetrated the leaf epidermis directly but occasionally entered through stomata. Recently, stomata were found to be important but not the only infection sites in the penetration and colonization of young cacao leaves (20,21).

Penetration of vegetative flushes of cacao by *C. perniciosa* has been observed only on leaves (7,20–22) even though petioles, pulvini, and stems are also susceptible to infection (7). Typical symptoms of witches'-broom were not obtained when leaves were inoculated with the agar block method (7), and thus it may be questionable that the penetration processes observed on leaves occur on the other parts of the vegetative flush.

Young flushes, in contrast to fully developed but unhardened flushes, seem to be more susceptible to infection by *C. perniciosa*. However, neither the optimal age of flushes for infection nor the reasons for reduced susceptibility with increasing age of the flush are known.

Observation of spore germination on plant surfaces, penetration mechanisms, and colonization of plant tissues by fungal pathogens is facilitated by the use of fluorescence microscopy (15,18,24). This technique has been used to study host responses to infection and how responses may be related to disease resistance (11,14). In this research, fluorescence microscopy was used to study germination of basidiospores of *C. perniciosa* on plant surfaces, the penetration mechanisms on the different parts of vegetative flushes, and the reaction of the host to infection. The objective of this study was to provide basic epidemiological information about the infection biology in order to develop standard inoculation techniques for evaluating resistance in cacao to the witches'-broom disease.

MATERIALS AND METHODS

Inoculum production and plant material. Diseased material (brooms) was obtained from Colombia, Venezuela, Brazil, and Trinidad. Brooms were placed in Plexiglas chambers in which dry and wet periods were regulated with humidifiers to stimulate the production of basidiocarps of *C. perniciosa* (16,17).

Cacao seeds from open-pollinated Catongo, Scavina 12, EET 19, and EET 233 clones were obtained from Costa Rica and Ecuador, planted in plastic pots (5 × 5 cm) containing Metromix 500 potting mix (Grace Horticultural Products, Cambridge, MA), and fertilized weekly with Peter's general purpose 20-20-20 fertilizer (Peters Fertilizer Products, Fogelsville, PA). Plants were grown for 2-6 mo at 21-30 C in a greenhouse with 50% shade provided by woven plastic shade cloth. Attached or detached vegetative flushes or flush parts were used at three stages of development described by Greathouse et al (10) as flushing 2 (F-2), interflush 1 (I-1), and interflush 2 (I-2). F-2 begins when expanding leaves become visible in the shoot tip and ends when the last expanding leaf of a growth flush is longer than 1.5 cm; I-1 follows F-2 and ends before all leaves of the flush attain the deep green of a mature leaf; and I-2 is when leaves are fully expanded and dark green.

Basidiospore germination and survival. Percentages of germination of basidiospores on cacao tissue were quan-

tified under different collection and incubation conditions. Leaves and stems from I-1 flushes of Catongo were placed on a piece of paper towel moistened with water in the bottom of a petri plate. A set of four leaves and four stems was sprayed with water to form a continuous film over their surfaces ("wet"), and another set was not sprayed ("dry"). Basidiocarps of *C. perniciosa* produced on brooms were stuck to the lid of a petri plate with petroleum jelly and placed over the stems and abaxial surfaces of leaves. Basidiocarps were removed after 5 min of active spore deposition. A third set of leaves and stems was sprayed with a suspension containing about 1 million spores per milliliter. The suspension was prepared by collecting basidiospores in a solution of 0.01 M MES at pH 6.1 and 0.01% Tween 20. Basidiospores were also collected on four dry slides to provide a comparison between germination in vitro and in vivo.

Each set of four inoculated plant parts and glass slides was incubated separately. One set was incubated at 25 C in a dew chamber, which produced and maintained a water film on the surfaces of plant parts and glass slides. The other three sets were incubated at room temperature (24–29 C) in closed plastic containers to which glycerol at a specific gravity of 1.137 or 1.082 or water was added to obtain a relative humidity (RH) of 80, 90, or 100%, respectively (5). After 6 hr of incubation, leaves and stems were removed from the dew chamber or the plastic containers; water was allowed to evaporate from the surface of the tissue, which was then sprayed with a solution of Cellufluor 0.03% in 0.01 M Tris/HCl buffer at pH 8. The samples were examined at ×125 and ×312 with a Leitz 20 microscope equipped with epifluorescence. A random sample of 200 basidiospores was examined to estimate the percentage of germination. This experiment was completed five times—twice with spores from basidiocarps produced on brooms from Venezuela and once each with those from Trinidad, Colombia, and Brazil.

Survival of basidiospores of *C. perniciosa* deposited for 5 or 25 min onto the dry surface of 10 leaves from Catongo I-1 flushes was studied. After spore deposition, one leaf from the 5- and 25-min exposures was sprayed with water and incubated at 25 C in a dew chamber for 24 hr; the other nine leaves of each group were separated into groups of three and incubated at room temperature (24–29 C) in plastic containers at 80, 90, or 100% RH. One leaf of each exposure period was removed from each container after 0.5, 1, and 2 hr, sprayed with water, and incubated in a dew chamber for 24 hr. Basidiospores were also deposited on wet plant surfaces to provide a comparison. Basidiospore germination was estimated after the procedure described

before. This experiment was completed five times—once each with inoculum from Brazil, Colombia, and Trinidad and twice with inoculum from Venezuela.

The ability of basidiospores to survive intermittent wet and dry periods was assessed. Basidiospores were deposited on five wet leaves from Catongo I-1 flushes. The inoculated leaves were incubated at 25 C in a dew chamber for 2–3 hr to allow formation of germ tubes one to two times the length of the basidiospore. The leaves were removed from the dew chamber to allow evaporation of water from their surfaces. After the surfaces were dry, the inoculated leaves were sprayed with water, returned to the dew chamber, and incubated for 24 hr. Cellufluor 0.03% was sprayed on the inoculated leaves, and the percentage of 200 basidiospores with germ tubes longer than the length of the spores was estimated on each leaf. The experiment was conducted twice using inoculum from Venezuela.

Germ tube development. Germ tube development of *C. perniciosa* on vegetative flushes of Catongo at different stages was examined to determine whether pathogen growth on plant surfaces occurred randomly or followed a pattern. Detached and attached F-2 and I-1 flushes of Catongo were placed horizontally in a dew chamber at 25 C. Five flushes of each developmental stage were sprayed with water and inoculated by placing basidiocarps on stems and the adaxial surfaces of leaves for 5 min. Hardened (mature, deep green) leaves from I-2 flushes were also inoculated. After 3, 12, and 24 hr of incubation, the inoculated tissue was removed from the dew chamber, kept in laboratory conditions to allow water to evaporate from

plant surfaces, and sprayed with 0.03% Cellufluor. Specimens were examined under the microscope with epifluorescence.

Germ tube growth on leaf surfaces was also studied with a method designed to avoid any disturbance of the position of germ tubes on the inoculated tissue. Basidiospores of *C. perniciosa* were collected in a solution containing 0.01 M MES buffer at pH 6.1, 15% glycerol, and 0.01% Tween 20. One drop of a 0.1% Cellufluor solution was added to every 5 ml of spore suspension and mixed for 10 min. The suspension was filtered through a 0.45-μm Millipore filter to remove the glycerol and excess Cellufluor. After filtering, the surface of the filter was kept moist to avoid spore damage. Basidiospores were washed off the filter with 0.2% agar; the resulting basidiospore suspension of unknown concentration was homogenized and sprayed onto five leaf pieces from Catongo F-2 and I-1 flushes placed on glass microscope slides. A coverslip was placed over the inoculated leaf pieces to facilitate observations under the microscope. Cellufluor did not affect basidiospore germination and was translocated to the germ tube; this allowed microscopic observations without further handling of the inoculated leaves. Observations were made after 3, 12, and 24 hr of incubation at 25 C in a dew chamber. This experiment was completed twice using inoculum from Colombia.

Host penetration and postpenetration development. Penetration mechanisms were observed on attached and detached leaves of F-2 and I-1 flushes and on hardened leaves of Catongo, EET 19, and EET 233. Basidiospores were deposited on all organs of the flush, as described

Table 1. Percentage of germination of basidiospores of *Crinipellis perniciosa* on cacao leaves according to inoculation method and incubation conditions

Inoculation method	Trial[w]	Basidiospore germination (%) with incubation conditions[x]			
		Dew	100% RH	90% RH	80% RH
Basidiospores deposited	1	0[y]	0	0	0
on dry surfaces	2	0	0	0	0
of leaves and slides	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
Basidiospores deposited	1	94	7.2[z]	0	0
on wet leaves	2	98	5.0	0	0
	3	100	1.3	0	0
	4	98	4.5	0	0
	5	98	7.0	0	0
Basidiospore suspensions	1	90	5.5	0	0
sprayed onto dry	2	91	4.0	0	0
surfaces of leaves	3	89	0.7	0	0
	4	83	7.9	0	0
	5	84	2.5	0	0

[w] Brooms from Trinidad, Brazil, and Colombia were used in trials 1, 2, and 3, respectively, and brooms from Venezuela were used in trials 4 and 5.
[x] Germination on stems was similar to that on leaves.
[y] Values are the average of 200 spores observed on five replications.
[z] Germinated basidiospores on leaves at 100% RH had only short germ tubes.
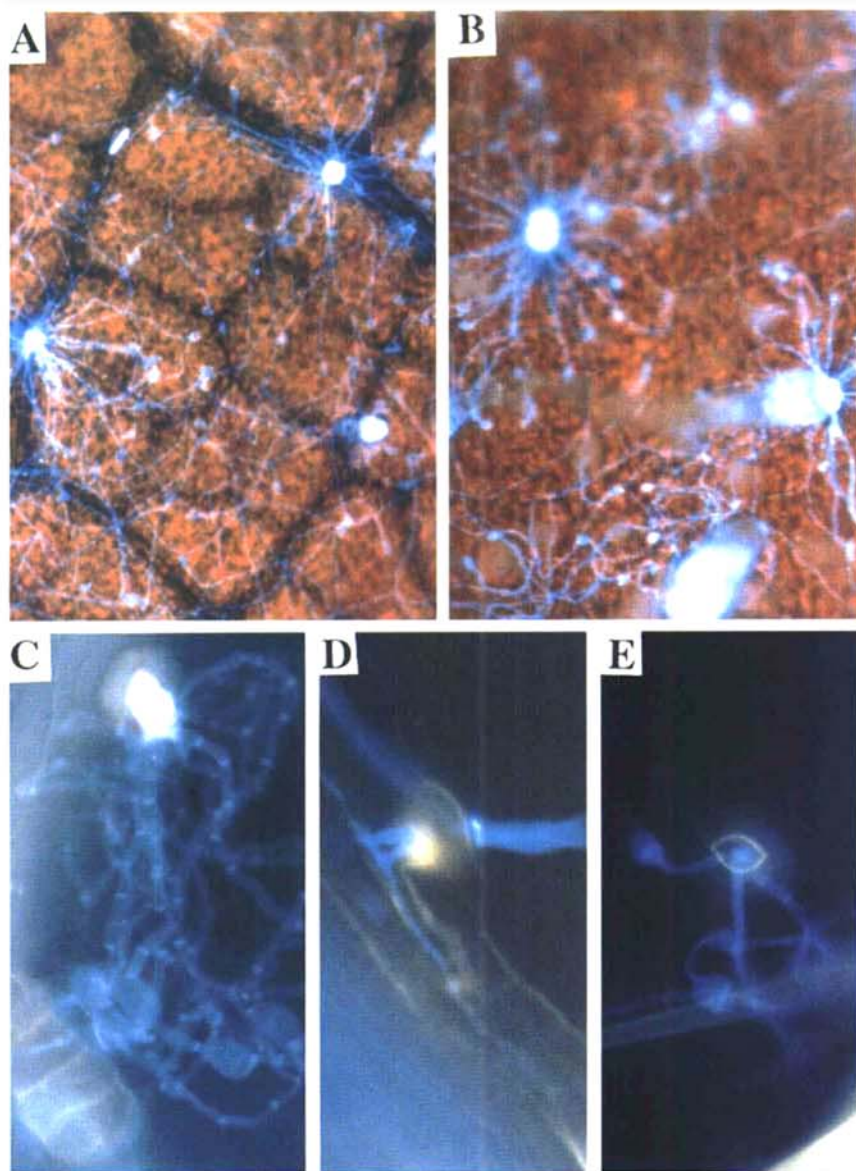
before, and incubated for 12, 24, and 48 hr at 25 C in a dew chamber. Some inoculated plants were transferred to the greenhouse and kept there for five additional days.

Inoculated leaves were cut into 1-cm² pieces and placed in chloroform/methanol (2:1) until chlorophyll was dissolved (10–15 min for young leaves). Leaf pieces were transferred to lactophenol/methanol (1:3), boiled for 10 min, and left overnight in this solution. Cleared leaves were washed twice for 15 min each with 100% methanol; passed through 10-min soakings of 95, 90, 80, 70, 50, 30, and 0% methanol in 0.01 M Tris/HCl buffer at pH 8.0; and transferred to a 0.1% solution of the fluorescence brightener Tinopal BHS in Tris/HCl buffer at pH 8.0 until mesophyll cells were observed with fluorescence microscopy (5–20 min). Specimens were washed twice for 5 min with Tris/HCl buffer and then placed in 1 M KOH at 60 C until leaves became transparent, which generally took from 4 hr for small leaves to overnight for fully developed leaves. The flaccid leaf pieces resulting from the KOH treatment were transferred first to water, then to glass microscope slides, mounted in lactophenol, and examined under fluorescence microscopy.

Fig. 1. Fluorescence photomicrographs of *Crinipellis perniciosa* germ tubes on cacao plant surfaces: (A) Directional growth of germ tubes toward stomata on veins of young leaf. (×125) (B) Directional growth toward trichomes on fully developed but nonhardened flushes. (×125) (C) Germ tubes entering stoma on a leaf 24 hr after inoculation. (×500) (D) Substomatal colonizing hyphae 7 days after inoculation. (×500) (E) Germ tube entering stoma on stem. (×500)
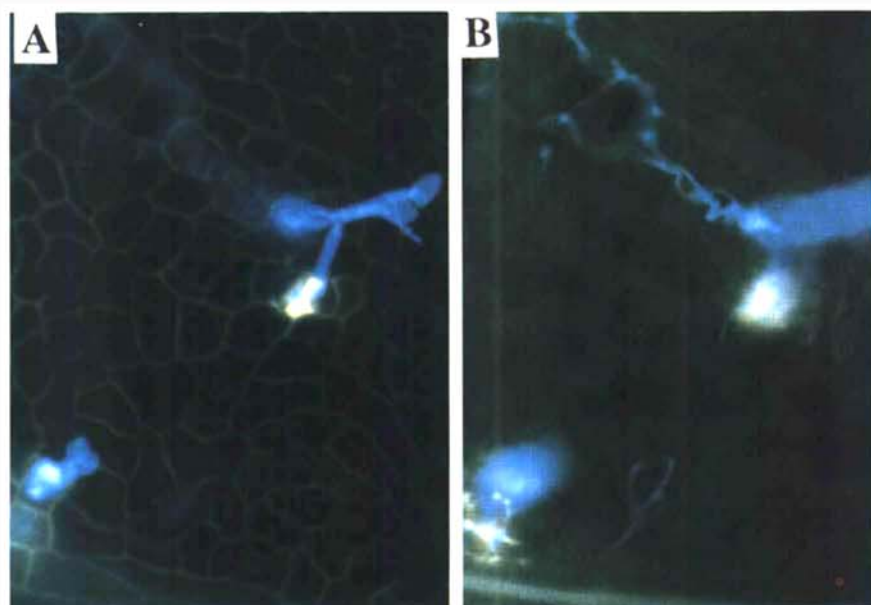

Fig. 2. Fluorescence photomicrographs of responses of cacao tissue to infection by *Crinipellis perniciosa* 48 hr after inoculation of a nonhardened leaf: (A) Three penetrated stomata and (B) mesophyll associated with same infection sites but with different degrees of golden fluorescence and growth of the pathogen within the leaf. Profuse hyphal growth at infection site without golden fluorescence contrasts with growth at sites with golden fluorescence. (×312)

Inoculated stems, petioles, and pulvini were soaked in a 0.1% solution of Tinopal for 5 min, then washed in buffer for 1 min. Freehand sections, cut perpendicular to the epidermis, were placed in 1 M KOH at about 60 C overnight, transferred to lactophenol, and left overnight or until tissues were clear. Tissue pieces were mounted in lactophenol for observation.

The ability of C. perniciosa to induce broom formation after leaf infection was evaluated by inoculating open-pollinated seedlings of Scavina 12 with axillary flushes bearing leaves no longer than 1.5 cm. Agar blocks with fresh basidiospore prints were applied to the abaxial surface of the leaves. Inoculated seedlings were incubated at 25 C in a dew chamber for 24 hr, then transferred to the greenhouse. They were kept in the greenhouse until symptoms developed and then were evaluated.

RESULTS

Basidiospore germination and survival. The percentage of germination of basidiospores in varying conditions of surface wetness is presented in Table 1. Basidiospores deposited on dry glass microscope slides or on dry host tissue for 5 min did not germinate regardless of subsequent treatments or incubation conditions. Germination occurred when basidiospores were deposited on wet flushes or when basidiospore suspensions were sprayed on dry surfaces of flushes and the flushes were placed in a dew chamber that provided optimal conditions for basidiospore germination. When inoculated wet plant parts were incubated at 100% RH, a low percentage of basidiospores began to germinate but did not develop further. Incubation at 90 and 80% RH did not result in any basidiospore germination. When the period of deposition on dry leaves was extended to 25 min and basidiospores were immediately incubated in a dew chamber, some germination occurred (about 5%). In the controls, germination occurred in 80–100% of the basidiospores deposited on wet leaves and incubated in a dew chamber.

Basidiospores did not survive intermittent wet and dry periods. Germ tube growth initiated on wet tissue was irreversibly terminated when the surface of the tissue was allowed to dry for 5 min. Cytoplasm of basidiospores that failed to germinate either on plant surfaces or on surfaces of glass microscope slides was condensed in the center of the cell; the space between the condensed cytoplasm and spore wall was apparently empty. These basidiospores appeared to be plasmolyzed.

Germ tube development. Germ tubes from basidiospores had a marked tropic response toward stomata on leaves and stems of young F-2 flushes, and many germ tubes were observed entering a single stoma (Fig. 1A). This tropic response was weaker on leaves from I-1 flushes and undetectable on fully hardened leaves. However, some germ tubes growing randomly on hardened leaves were also observed entering stomata. Germ tubes were also attracted toward the bases of fallen or collapsing multicellular trichomes on fully developed but nonhardened I-1 flushes (Fig. 1B). No differences were evident between attached and detached flushes regarding growth of the germ tubes toward the penetration sites.

Host penetration and postpenetration development. Penetration of cacao tissue by germ tubes of C. perniciosa occurred through stomata on leaves (Fig. 1C) and stems (Fig. 1E). Penetration was observed on all parts of young F-2 flushes and on fully developed but nonhardened I-1 flushes. No appressoria or other specialized penetration structures were observed on stomata through which the germ tubes entered (Figs. 1C and 2A). Penetrating germ tubes increased in diameter in the substomatal cavity and resembled vesicles. Intercellular hyphae developing from these vesicles increased from sparse to abundant 1–7 days after inoculation (Fig. 1D).

Germ tubes penetrated fully developed but nonhardened I-1 flushes through the bases of fallen or collapsed multicellular trichomes. Vesicles developed after penetration, but subsequent colonization was not observed. On fully hardened leaves, germ tubes were observed entering stomata, but neither substomatal vesicles nor colonizing hyphae were observed. No differences in the penetration mechanisms were evident between attached and detached flushes.

Growth of C. perniciosa after penetration varied among infection sites in the same flush and within the same leaf. In some instances, hyphae grew freely throughout the infected tissue; in others, mycelial growth stopped shortly after penetration. This failure to colonize cacao tissue was associated with the development of a golden fluorescence of host cells at and around the sites of penetration (Fig. 2). The intensity of this fluorescence varied from site to site and was apparently correlated negatively with the extent of colonization 24 and 48 hr after inoculation. However, 7 days after inoculation, many of the successfully colonized infection sites also showed some degree of golden fluorescence (Fig. 1D). The frequency of penetrated stomata that developed golden fluorescence was higher in leaves from young F-2 flushes of EET 233 than in leaves of Catongo or EET 19. No attempt was made to quantify this observation.

Inoculation of leaves on young F-2 flushes resulted in broom development. Nine of 16 of the inoculated flushes of Scavina 12 developed typical symptoms of witches'-broom. The petiole and pulvini of the infected leaf were always swollen. In many cases, the axillary bud closest to the inoculated leaf developed into a broom, and the hypertrophy did not extend below the petiole of the inoculated leaf.

DISCUSSION

Basidiospores lost viability immediately after contacting the dry surface of cacao flushes or glass slides. The cytoplasm concentrated near the centers of these basidiospores caused the spores to resemble plasmolyzed cells, a condition that might have resulted from a loss of water by the spores. The cause of the cytoplasmic disorganization was not determined, however. Prolonged deposition of basidiospores on dry leaves or glass slides (5–25 min) allowed a low percentage of basidiospores to survive, probably because layers of basidiospores closer to the dry surface protected subsequently deposited spores from the adverse effect of the dry surface. Basidiospore germination required free water on the surface of the tissue. When wet inoculated plant parts were incubated at 80–100% RH and room temperature (24–29 C), surface water evaporated soon after inoculation because the dew point (20–23 C) was never reached (5). Water on the plant parts incubated at 100% RH took longer to evaporate, which explains the low percentage of basidiospores that initiated germination but did not develop further (Table 1). These observations have important implications for the efficiency of inoculation methods. For example, a high proportion of basidiospores collected on dry glass surfaces lose viability. When this collection method is used to prepare inoculum, the concentration of viable basidiospores will be reduced considerably. Basidiospore germination, host penetration, and the establishment of infection are irreversibly terminated if the plant surface where the basidiospore is located becomes dry before infection is established. Therefore, inoculated plants surfaces must be kept wet until an infection is established.

Basidiospores or germ tubes of C. perniciosa were not able to survive for any period of time on dry flushes. The stringent requirements for liquid water on any surface as a prerequisite for germination are met in nature, since the peak of basidiospore release occurs between 2200 and 0400 (2,8,19), when dew is prevalent. Basidiospores released at other times during the day have a reduced probability for infection. Those released early in the morning (0400–0600) may land on plant surfaces wet by dew, but the dew may not persist for the time required for germination and penetration. Also, basidiospores released during the hours of 0700–1800 may encounter rising temperatures and evaporation of free water (from dew or rain) from the surface of the plant before infection

is established.

The evident tropic response of germ tubes of *C. perniciosa* increases the chances for infection. The intensity of this tropic response decreased as flush development progressed. Perhaps the reduction in carbohydrate availability during the development of cacao flushes (12) may produce changes in the quality and quantity of exudates at the infection sites and, thus, affect the intensity with which they attract germ tubes of *C. perniciosa*.

Basidiospores of *C. perniciosa* germinated and penetrated through stomata on all organs of nonhardened flushes, and substomatal vesicles and colonizing hyphae from them were observed in every part of the flush. Thus, stomata were the infection sites on vegetative flushes. This infection mechanism is remarkably similar to that reported by Patton and Johnson (15) on white pine needles inoculated with *Cronartium ribicola* J.C. Fisch. The role of substomatal vesicles of *C. perniciosa* in the infection process was not studied, but they were common 12 hr after inoculation, suggesting that subsomatal vesicles may protect the pathogen from desiccation in recently established infections. Wetness on plant surfaces may be essential for the infection process only until substomatal vesicles are formed. On hardened leaves, substomatal vesicles and colonization hyphae were never observed. Probably, the nutritional requirements of the pathogen for postpenetrational growth are not met in this type of tissue.

Colonizing hyphae were not observed to develop from penetrated multicellular trichome bases. However, a strong attraction of germ tubes toward trichome bases occurred on fully developed but nonhardened I-1 flushes (Fig. 1B). Trichome bases may be important ports of entry for the pathogen before flush hardening. Although colonization of I-1 flushes apparently does not occur soon after penetration, vesicles formed under the epidermis could remain dormant until the next flush cycle. The position of these dormant infections would probably determine the type of symptoms that would develop. Infections relatively distant from the apical meristem may produce swellings or cankers; brooms could be produced when the infections occur close to axillary or apical buds.

Direct penetration was never observed in this study. Other researchers have observed germ tubes of *C. perniciosa* penetrating the leaf epidermis directly (7,21). Penetration through small wounds produced on the leaves during processing for inoculation (surface-sterilized) could result in apparent direct penetration.

Infection sites (stomata) within a flush were not colonized equally, and colonization 24–48 hr after inoculation apparently decreased as the intensity of the golden fluorescence at and around the penetrated stomata increased. This fluorescence correlated with resistance to witches'-broom in open-pollinated seedlings of EET 233 (resistant to witches'-broom) that had a higher frequency of golden fluorescence associated with stomata penetrated by germ tubes than open-pollinated seedlings of EET 19 (susceptible to witches'-broom). In other crops, host resistance to certain diseases has been correlated positively with the proportion of infection sites in which a similar golden fluorescent reduction was observed after penetration (9,11,14).

Successfully colonized infection sites that showed a golden fluorescence 7 days after inoculation could be the result of a delayed response of the plant to infection. However, the importance of this delayed response on subsequent colonization was not established. The nature of the golden fluorescence was not elucidated, but certain phenolic compounds that may be responsible for this type of fluorescence have been extracted from cacao tissues and have inhibited germination of basidiospores of *C. perniciosa* (4).

Inoculation of leaves (5–15 mm long) on young flushes gave rise to the development of brooms. Cronshaw and Evans's (7) lack of success in obtaining brooms from leaf inoculation may have been due to the age of the leaves or the type of flush used, or both. The efficiency of inoculation methods using vegetative flushes could be improved by covering all parts of the flush with inoculum rather than applying basidiospores to specific sites, as some inoculation methods require (3,6,7).

A standardized method for inoculation of cacao with *C. perniciosa* requires that plant surfaces be kept wet during the incubation period. Also, to evaluate resistance to witches'-broom, the inoculum concentration must be known and controlled.

LITERATURE CITED

1. Aragundi, J. A. 1982. Culture and histology of *Crinipellis perniciosa* (Stahel) Singer. M.S. thesis. University of Florida, Gainesville. 52 pp.
2. Baker, R. E. D., and Holliday, P. 1957. Witches' broom disease of cacao (*Marasmius perniciosa* Stahel). Phytopathol. Pap. 2. Commonw. Mycol. Inst., Kew, Surrey, England. 42 pp.
3. Baker, R. E. D., and McKee, R. K. 1943. Witches' broom investigations. VI. The infection of flower cushions and pods of cacao by *Marasmius perniciosus* Stahel. Trop. Agric. 20:188-194.
4. Bastos, C. N. 1987. Inhibitory effect of extracts of cocoa cultivars and phenolic compounds on basidiospore germination of *Crinipellis perniciosa*. Pages 371-374 in: Proc. Int. Cocoa Res. Conf. 10th.
5. Braun, J. V., and Braun, J. D. 1958. A simplified method for preparing solutions of glycerol and water for humidity control. Corrosion 14:17-18.
6. Briton-Jones, H. R., and Cheesman, E. E. 1931. Witch broom control. Trop. Agric. 8:79-89.
7. Cronshaw, D. K., and Evans, H. C. 1978. Witches' broom disease of cocoa (*Crinipellis perniciosa*) in Ecuador. II. Methods of infection. Ann. Appl. Biol. 89:193-200.
8. Evans, H. C., and Solorzano, G. R. 1982. Witches' broom disease: Wrong experiment, right results. Pages 415-418 in: Proc. Int. Cocoa Res. Conf. 8th.
9. Gangadin, S. 1987. Differentiation of cultivars of sugarcane by their resistance reaction to *Puccinia melanocephala*. M.S. thesis. University of Florida, Gainesville. 52 pp.
10. Greathouse, D. C., Laetsch, W. M., and Phinney, B. O. 1971. The shoot-growth rhythm of a tropical tree, *Theobroma cacao*. Am. J. Bot. 58:281-286.
11. Luke, H. H., Barnett, R. D., and Pfahler, P. L. 1984. Postpenetration development of *Puccinia coronata avenae* in slow- and fast-rusting cultivars of *Avena byzantina*. Phytopathology 74:899-903.
12. Machado, R. C., and Hardwick, R. 1987. Dynamics and histology of individual leaf and whole flush development yields clues on the control of cocoa flush cycle. Pages 143-149 in: Proc. Int. Cocoa Res. Conf. 10th.
13. Medeiros, A. G. 1975. Liberacao e longevidade de basidiosporos da *Marasmius perniciosus*. Pages 62 and 89 in: Inf. Tec. 1974.
14. Niks, R. E., and Kuiper, H. J. 1983. Histology of the relation between minor and major genes for resistance of barley to leaf rust. Phytopathology 73:55-59.
15. Patton, R. F., and Johnson, D. W. 1970. Mode of penetration of needles of eastern white pine by *Cronartium ribicola*. Phytopathology 60:977-982.
16. Rocha, H. M. 1983. The ecology of *Crinipellis perniciosa* (Stahel) Singer in witches' broom on cacao (*Theobroma cacao* L.). Ph.D. thesis. University of London. 145 pp.
17. Rocha, H. M., and Wheeler, B. E. J. 1982. The water balance as an important factor in basidiocarp production by *Crinipellis perniciosa*, the causal fungus of cocoa witches' broom. Pages 381-386 in: Proc. Int. Cocoa Res. Conf. 8th.
18. Rohringer, R., Kim, W. K., Samborski, D. J., and Howes, N. K. 1977. Calcofluor: An optical brightener for fluorescent microscopy of fungal plant parasites in leaves. Phytopathology 67:808-810.
19. Solorzano, G. R. 1977. Factores involucrados en la produccion de basidiocarpos y en la diseminación de basidiosporas de *Marasmius perniciosus* Stahel. Ing. Agr. thesis. Universidad de Manabi, Ecuador.
20. Sreenivasan, T. N. 1987. The pernicious nature of the witches' broom pathogen. Abstr. 83 in: Summaries Int. Cocoa Res. Conf. 10th.
21. Sreenivasan, T. N., and Dabydeen, S. 1989. Modes of penetration of young cocoa leaves by *Crinipellis perniciosa*. Plant Dis. 73:478-481.
22. Stahel, G. 1932. Contribution to the knowledge of witchbroom disease. (Dep. vanden Landbow Surinam Bull. 39, 1919; translation from Dutch.) Trop. Agric. 9:167-176.
23. Suarez, C. 1977. Growth of *Crinipellis perniciosa* (Stahel) Singer in witches' broom on cocoa (*Theobroma cacao* L.). Ph.D. thesis. University of London. 134 pp.
24. Yigal, C., Pe'er, S., Balass, O., and Coffey, M. D. 1987. A fluorescent technique for studying growth of *Peronospora tabacina* on leaf surfaces. Phytopathology 77:201-204.