Resistance

Compartmentalization of *Ceratocystis fagacearum* in Turkey Oak in South Carolina

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ABSTRACT

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Nine turkey oak trees were inoculated on 30 April 1980 with *Ceratocystis fagacearum*. Although the trees partially wilted 4–6 wk later, when harvested on 16 August 1982, *C. fagacearum* was not recovered and the trees were still alive. A CODIT (compartmentalization of decay in trees) reaction was associated with infection. Tylosed vessels and densely stained vertical parenchyma cells formed noncoalescing visible discolorations mainly in 1980 xylem tissues. Increased tylose formation and discoloration, evident in crowns, stems, and roots the year of inoculation, subsequently decreased. Tyloses were present in fewer than 50% of the vessels, indicating a limited physiological effect of infection on this host. The CODIT reaction apparently plays an important role in South Carolina by limiting survival of *C. fagacearum* and enhancing the recovery of infected red oak hosts.

In South Carolina, natural occurrence of the oak wilt fungus (*Ceratocystis fagacearum* (Bretz) Hunt) is relatively sparse. Although present in the state since at least 1968 (12), *C. fagacearum* has had so little impact on the state's oak timber resources that detection and impact surveys are no longer conducted.

Tainter and Ham (11) observed that artificially inoculated turkey oaks (*Quercus laevis* Walt.) were not quickly killed by *C. fagacearum*. Although many trees died, some trees not only survived the inoculation and wilting but apparently recovered totally. The fungus was not isolated from these trees 2 yr after inoculation, at which time stems, most major branches, and roots were still alive and showed no evidence of active wilting. Vascular discoloration, however, was evident (Fig. 1A). The authors (11) speculated that high summer temperatures in the inoculated trees debilitated *C. fagacearum* to the extent that only a weak wilt reaction resulted.

The purpose of the present research was to examine the vascular discoloration and to detect histological changes that had occurred in a sample of the trees still alive about 2 yr after artificial inoculation with *C. fagacearum*.

MATERIALS AND METHODS

The trees examined in this investigation were part of previous research in which the study area, methodology, and results were reported (11). In that study, 250 trees of *Q. laevis* growing in the Sand Hills State Forest, South Carolina, were inoculated with a South Carolina isolate of *C. fagacearum* on 30 April 1980. During the subsequent 405 days, trees were sampled and various physiological conditions, including moisture content, were measured and isolation of *C. fagacearum* was attempted.

On 16 August 1982, 808 days after these trees were inoculated, a final survey was conducted. Many trees that were inoculated in 1980 remained alive in 1982. Eighteen trees (nine inoculated and nine control) were randomly selected and harvested for isolation of *C. fagacearum* and histological examination.

Trees were felled, and cross-sectional disks 2–4 cm thick were removed at 0.25 and 1.4 m above ground. Four living branches 1–2.5 cm in diameter from the outer crown of each tree were collected. In addition, the root systems were excavated and four separate roots were sampled from each tree. Isolations were made by removing xylem chips from alcohol-dipped and flamed samples, then plating chips onto potato-dextrose agar to recover *C. fagacearum*. Larger samples were removed with a band saw, fixed in formalin-acetic acid-alcohol, and processed through a dehydrating series (2) in which *n*-butyl alcohol replaced tertiary butyl alcohol, then into Tissuemat at 62 C.

Cross sections and longitudinal sections 20 μm thick were prepared with a sliding microtome. Samples were viewed either unstained or stained with safranin and counterstained with fast green (2). One section was selected from each tissue sample and viewed in detail with light microscopy, and photomicrographs were prepared. Within each annual ring or increment, the total number of vessels was recorded and vessel diameter measured parallel with the cambium. The presence or absence of tyloses in each vessel ≥ 10 μm in diameter was recorded.

Linear, straight-line functions of the form $y(x) = a + bx$ were adopted to characterize the relationship between vessel diameter (concommitant variable x, range 10–28 μm) and percent tylose formation (response variable y), and regression lines were fitted using weighted least-squares estimation. Comparison of the regression lines for control and inoculated trees was conducted in a manner similar to that described by Neter and Wasserman (4).

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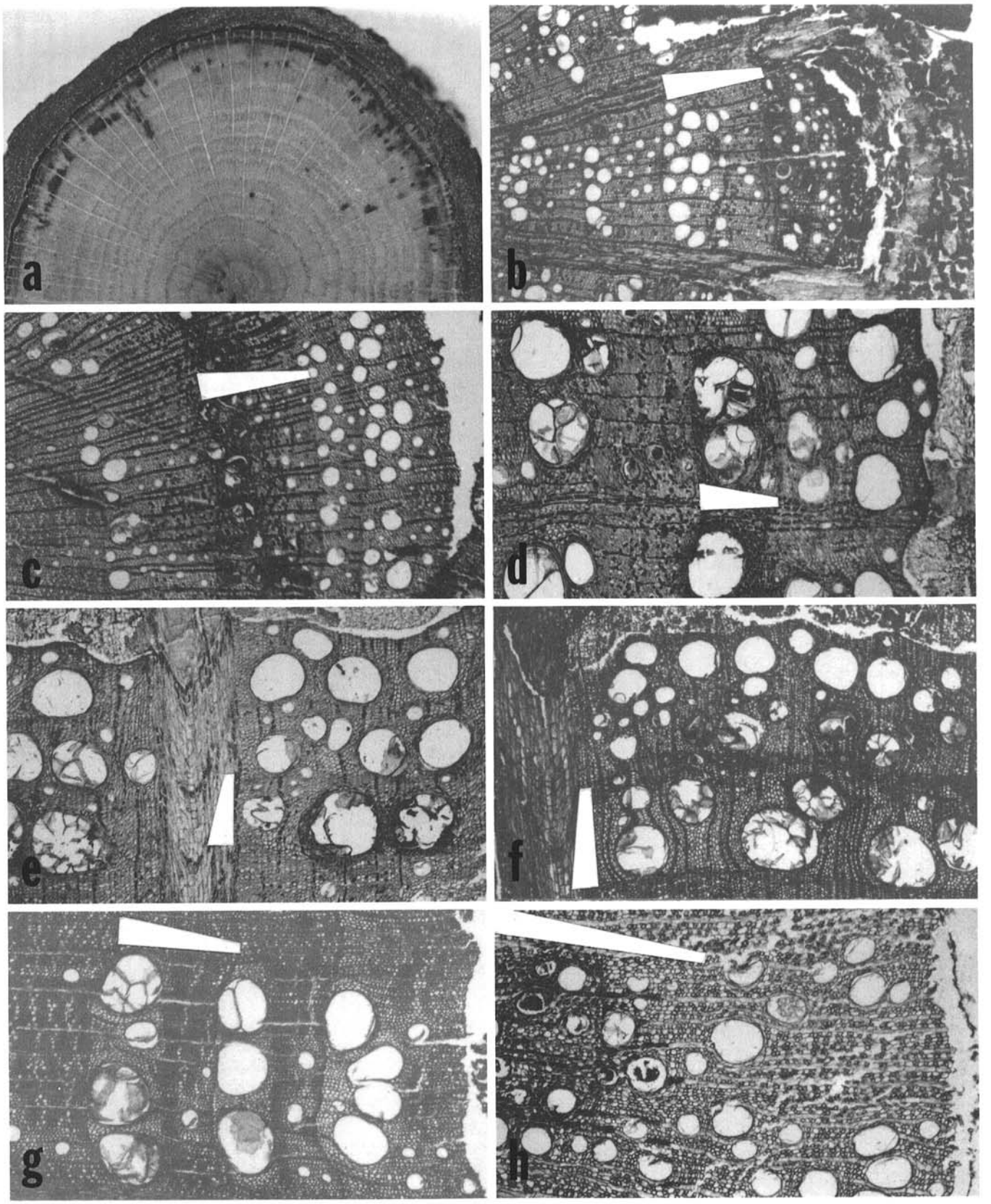

Fig. 1. A, Stem of turkey oak showing vascular discolorations resulting from infection by *Ceratocystis fagacearum*. **B,** Discoloration sector in 1981 annual increment of branch. **C,** Continuous discoloration band in 1980 branch increment showing tylosed vessels and darkly stained apotracheal parenchyma and scattered darkly stained vertical parenchyma in summerwood of 1979 increment. **D,** Discolored strand of xylem in upper stem with tylosed vessels and densely stained paratracheal parenchyma in 1980 increment and tyloses and densely stained vertical parenchyma in latewood of 1979 increment and some tylose formation in same year. **E,** Discolored strand in upper stem with tylosed vessels in 1980 increment (note relative absence of densely stained vertical parenchyma and only limited darkly stained paratracheal parenchyma). **F,** Lower stem with tylosed vessels in 1980 and 1981 annual increments showing absence of darkly stained vertical parenchyma. **G,** Upper stem with tyloses in 1980 and 1981 increments and no evident discoloration. **H,** Root with discolored zone in 1980 increment showing densely stained vertical parenchyma and few tylosed vessels. All sections are at ×50 except A, which is actual size. Arrows point toward the cambium and mark the location and width of 1980 growth. Growth rings for 1981 and 1982 can be identified by the band of relatively large springwood vessels in each.

RESULTS

Discolored xylem was present in the 1980 annual rings of branches, upper and lower stems, and roots of inoculated trees, providing indirect evidence that the pathogen had spread great distances vertically within the xylem. This response and the presence of discolored wood and patterns of host reaction associated with infections of *C. fagacearum* in *Q. laevis* appeared to fit the CODIT (compartmentalization of decay in trees) model (9).

Statistical analysis of the two straight-line models for the relationship between vessel diameter and percent tylose formation in inoculated and control trees indicated no difference between the slopes of the models; however, the percentage of vessels containing tyloses was consistently higher in inoculated trees (Fig. 2).

The percentage of vessels with tyloses was also greater for all sampled positions within inoculated trees than within control trees. Disease-associated tylose formation increased in aboveground portions of trees in 1980, the year of inoculation (Fig. 3). Relative percentages of vessels with tyloses subsequently decreased to normal levels in upper and lower stems during the following 2 yr. In branches, the decline of tylose formation in 1981 and slight increase in 1982 may represent effects of residual surviving infections in some branches. In aboveground portions of these trees, there was also a disease-related increase of tylose formation in pre-1980 annual growth, especially in 1979 within the upper stem. Tylose formation in roots increased to 30.2% in 1980 and then to 47.8% in 1981 before declining (Fig. 3). This pattern of tylose formation in roots of diseased trees suggests a 1-yr lag effect caused by infection by *C. fagacearum*.

Visible host reaction to infection was limited to long but tangentially and radially localized, discolored strands of xylem tissue. The pathogen was only occasionally able to kill the cambium (Fig. 1B).

Visible discolorations were mostly composed of tyloses and associated gums in vessels and were often, but not always, associated with darkly stained vertical parenchyma. Discolorations in cross section were usually noncoalescing (Fig. 1A,D,E,H).

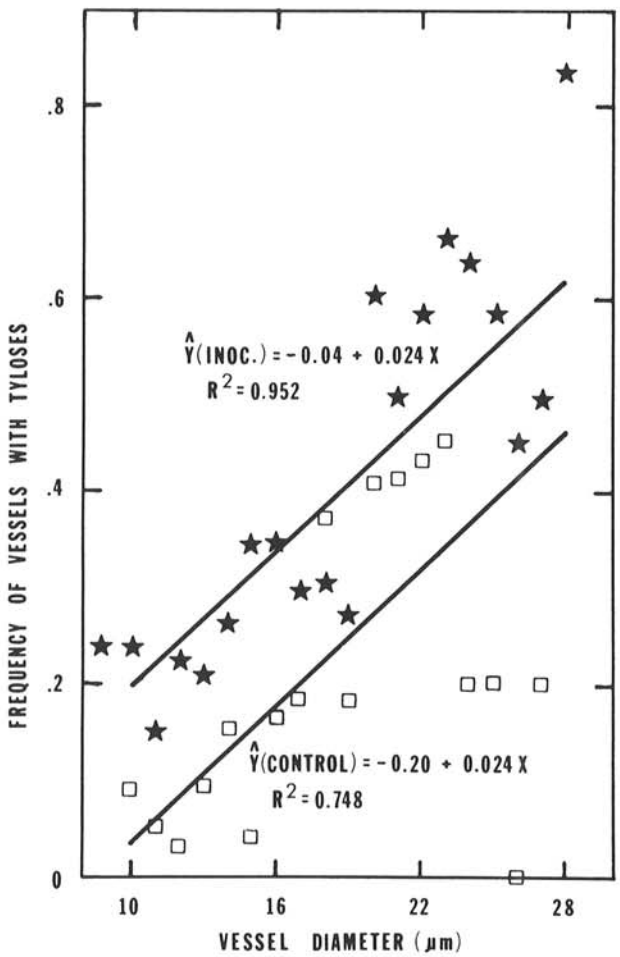

Fig. 2. Tylose formation in turkey oak trees inoculated with *Ceratocystis fagacearum* and in uninoculated control trees.

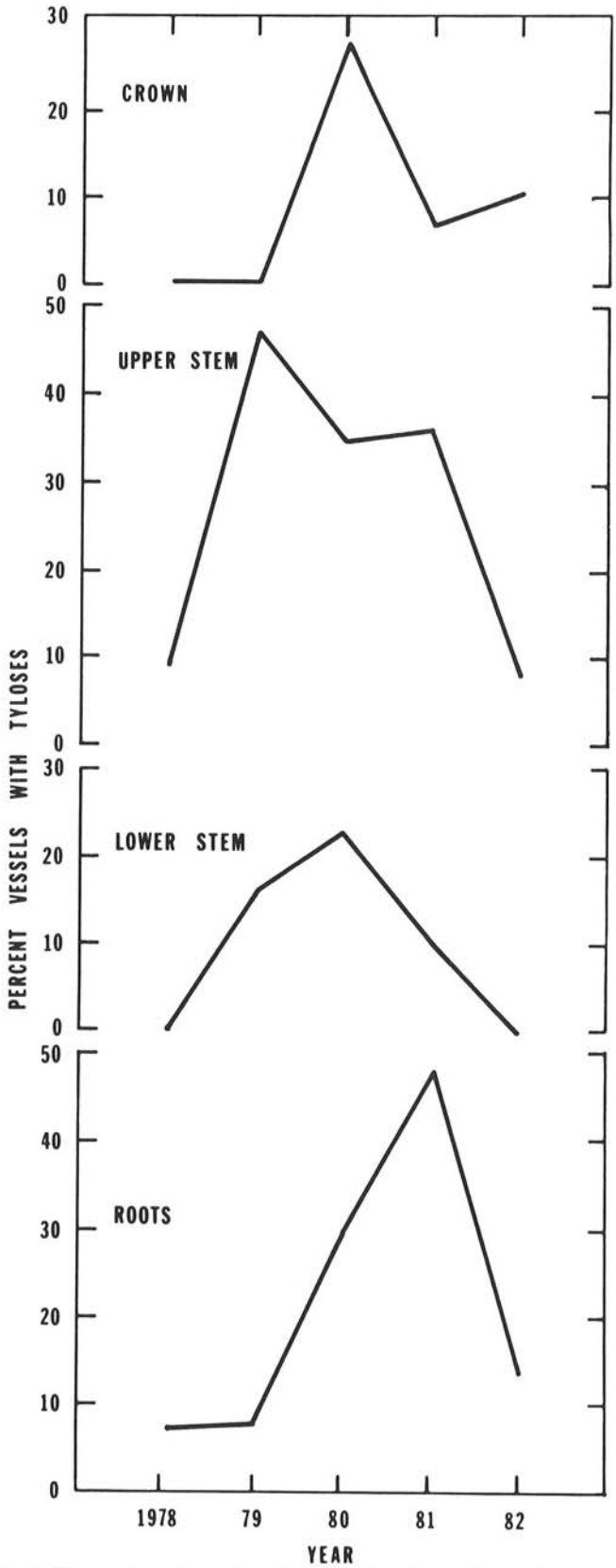

Fig. 3. Disease-dependent tylose formation in turkey oak trees inoculated with *Ceratocystis fagacearum* on 30 April 1980 and adjusted for normal tylose formation in control trees.

Occasionally, discolorations spread into the 1979 growth ring (Fig. 1C,D) or into the 1981 and/or 1982 growth rings (Fig. 1B,F,G).

Discolored areas often involved only a few vessels (Fig. 1D) but sometimes spread tangentially across rays to incorporate several dozen vessels or to form a continuous tangential line of discoloration in the annual increment (Fig. 1C). More frequently, a smaller sector of xylem bounded on either side by rays was discolored and contained vessels with tyloses (Fig. 1B).

Presence and location of densely staining vertical parenchyma was irregular. Discolored areas of xylem sometimes contained tylosed vessels surrounded by densely stained paratracheal or apotracheal parenchyma, but occasionally in roots, discolored vertical parenchyma was associated only with tylose-free vessels (Fig. 1H).

DISCUSSION

Shigo (9), in his CODIT concept, recognizes four barriers to infection in woody tissues: wall 1, which resists vertical spread of the pathogen by the formation of plugging walls; wall 2, which resists the inward spread of the pathogen by a type of latewood formation; wall 3, which consists of rays that resist lateral spread; and wall 4, which resists infection into subsequently formed xylem. This last wall is an inherent property of the cambium that can be further augmented by formation of resistant cell types and toxic chemical deposition in response to injury or infection.

C. fagacearum initially invades the xylem vessels of the outer sapwood (7). Later, in a susceptible host, hyphae penetrate the adjacent xylem parenchyma through the pits. In the *Q. laevis*-*C. fagacearum* model described here, it appears that all the CODIT walls are operative during early infection and restrict growth of *C. fagacearum* from the vessels. Springwood had already been formed by April, when these trees were inoculated. Therefore, the tyloses present in these vessels and the associated discoloration in surrounding cells would be direct evidence of wall 1. The tylosed vessels and associated densely stained vertical parenchyma produced a distinctly visible vascular browning. This is similar to the reaction occurring in *Q. macrocarpa* Michx. infected with *C. fagacearum* (5,7). In *Q. alba* L. and *Q. prinus* L., there is also an intense tylose development and formation of a darkly stained zone of paratracheal parenchyma cells in early stages of infection (1). In *Q. alba* (1) and *Q. macrocarpa* (7), hyphal growth ceases after early infection as electron-dense material forms within invaded paratracheal parenchyma cells. This material appears to restrict the fungus, and in these species, the cambium is able to form new wood over the infected wood and the tree recovers. In susceptible *Q. rubra* L., hyphae and conidia increase with time as the fungus spreads laterally into many small vessels and tracheids (1). These cells lack associated parenchyma cells and, therefore, are unable to produce pathogen-resisting tyloses and gums. A susceptible host such as *Q. rubra* soon wilts and dies in response to infection.

Wall 2, or latewood formation in *Q. laevis*, was certainly effective because infection seldom spread into the annual rings of the previous or succeeding year's growth. In *Q. macrocarpa* (5), there was a small amount of vascular browning in the preceding annual ring but there was no outward extension of the stain beyond the infected ring.

Wall 3, consisting of the rays, was evident in *Q. laevis*, appearing as wedges of vascular browning bounded by rays on two sides.

We interpret the increased incidence of discolored vertical parenchyma in latewood as evidence of wall 4. This wall, however, can be present in a number of forms, ranging from simply a lining of suberin in cambial cells (9) that renders them very resistant to microbial invaders to the creation of fewer vessels and fibers and more parenchyma cells, which have the ability to produce toxic phenolics. A contiguous reaction zone in *Q. macrocarpa* (3) and *Q. alba* (8) consisting of vascular browning and tylose formation was interpreted (10) as a barrier zone. This more reactive zone was not visible in *Q. laevis*.

Oak-wilted trees in South Carolina do not always recover. Of those that do ultimately die, the death process more nearly resembles a decline syndrome resulting from depleted energy reserves. *Hypoxylon atropunctatum* (Schw. ex Fr.) Cke., a common invader of stressed or recently dead red oaks, does not produce noticeable sapwood decay or fruiting bodies on these oak-wilted trees until they are in an advanced stage of decline or have died (11).

South Carolina is on the southeastern periphery of the known range of oak wilt (6). Why this disease does not occur farther south has long been an enigma to forest pathologists. Because both naturally and artificially inoculated red oaks of several species in South Carolina, including *Q. laevis*, often survive for several years, or even recover, and there seems to be little natural spread to adjacent trees, one could conclude the significant involvement of effective host resistance mechanisms. The CODIT reaction described in the present research seems to be one example of an effective host resistance mechanism. We believe this is the first description of a CODIT resistance mechanism to *C. fagacearum* in a red oak species. Because the host is not quickly killed, it is able to react to infection by the CODIT mechanism. As a result, many infections do not survive and trees recover.

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