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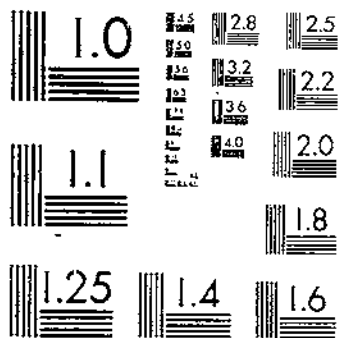
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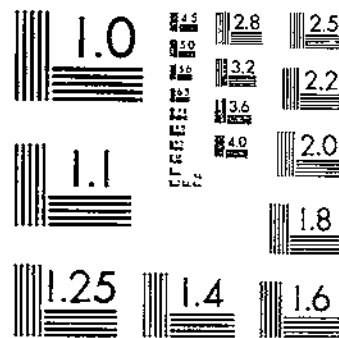
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CAMPTOTHECA ACUMINATA DECAISNE (NYSSACEAE) SOURCE OF CAMPTOTHEGIN  
PERDUE, R. E., ET AL. 1 OF 1

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MICROCOPY RESOLUTION TEST CHART  
NATIONAL BUREAU OF STANDARDS-1963-A



MICROCOPY RESOLUTION TEST CHART  
NATIONAL BUREAU OF STANDARDS-1963-A

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Decaisne (Nyssaceae)  
Source of Camptothecin,  
an Antileukemic Alkaloid

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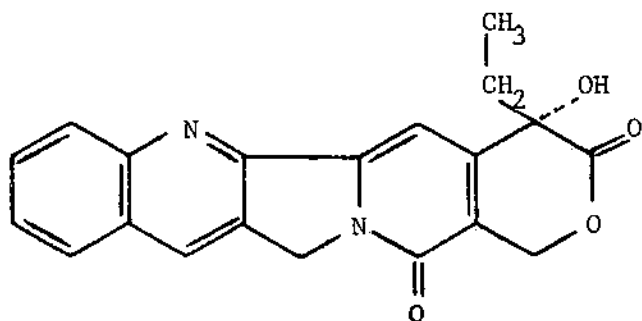
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***Camptotheca acuminata* Decaisne (Nyssaceae)**  
**Source of Camptothecin, ;**  
**an Antileukemic Alkaloid**

By ROBERT E. PERDUE, JR., botanist, and ROBERT L. SMITH, horticulturist, Crops Research Division, Agricultural Research Service; MONROE E. WALL, Director, Chemistry and Life Sciences Laboratory, Research Triangle Institute; and JONATHAN L. HARTWELL, chemist, and BETTY J. ABBOTT, biologist, Cancer Chemotherapy National Service Center, National Cancer Institute

*Camptotheca acuminata* Decaisne was introduced into the United States from China by the U.S. Department of Agriculture in its continuing plant introduction program. Extracts from samples of this tree, provided by the U.S. Plant Introduction Station, Chico, Calif., were tested in the antitumor screening program of the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute. These tests demonstrated that crude extracts of *C. acuminata* have significant activity against lymphoid leukemia L-1210 in mice.

The chemical agent responsible for the antileukemic activity was isolated and characterized at the Research Triangle Institute, Durham, N.C. This agent is camptothecin, a new alkaloid (25, 27).<sup>1</sup> The following diagram shows its unusual chemical structure.



Camptothecin is present in minute quantities in all parts of the tree. Several other compounds related to it, but found only in trace quantities, were also isolated from *C. acuminata*. They display similar biological activity (26).

*C. acuminata* is only one of several hundred plant species that have shown activity against one or more tumor systems used in the CCNSC primary screen. It is, however, one of the more spectacular

<sup>1</sup> Italic numbers in parentheses refer to Literature Cited, p. 25.

because of its unique history and its activity against lymphoid leukemia L-1210. L-1210 appears to have a higher clinical predictability value than most other tumor systems employed in the antitumor screening program (9).

Camptothecin has successfully passed only the first of several stages that lead to the acceptance of a natural product as a clinical drug. Pharmacological evaluation in dogs and other laboratory animals must demonstrate that it is unlikely to produce unacceptable side effects. The efficacy of the drug must also be demonstrated in human patients.

Chemists are attempting to synthesize camptothecin. If their effort is unsuccessful or synthesis proves less economical than isolation from the natural source, American agriculture can produce almost unlimited quantities of raw material. *C. acuminata* is another example of the potential rewards to be gained from the introduction of foreign plant species and coordinated botanical, chemical, and biological evaluation of our plant resources.

This bulletin includes all available information about the tree. The Chinese literature was reviewed by H. L. Li, Morris Arboretum, University of Pennsylvania, Philadelphia. No medicinal value was attributed to *C. acuminata*. "In fact, the plant is rare in China and little mentioned in the literature. In one work where it is mentioned, it is noted specifically as something of no particular value."<sup>2</sup>

### Botany

The common names for *Camptotheca acuminata* are as follows: Hsi shu, tree of joy (18); han lien mu, dry lotus tree (Mt. Omei, Szechwan); shu li tze, waterchestnut (Chiu Li, Szechwan); chien chang shu, thousand-sheet tree (Szechwan); tien tzu shu, heaven-wood tree (Hunan); and hsi hsu, tree of love (Nanking, Kiangsu).<sup>3</sup>

### Taxonomy

*C. acuminata* was described by Joseph Decaisne from specimens collected by Armand David (6). The type collection was from Lü Shan, a mountain just south of Kiukiang in northernmost Kiangsi Province, China.

*C. yunnanensis* Dode differs but slightly from *C. acuminata*. The name was probably based on an immature fruiting specimen (31). Dode's name has not been accepted by any author reporting on the flora of China.

*C. crassa* C. and E. M. Reid (16) was applied to a European fossil fruit later considered to be a fossil *Halesia* (10).

The genus name is from the Greek—*campto* (bent or curved) and *theca* (a case) in reference to the anthers, which are bent inward.

<sup>2</sup> Li, H. L., personal communication, Oct. 29, 1968.

<sup>3</sup> Kiang, T., Forestry Div., Joint Comm. on Rural Reconstruction, Taipei, Taiwan, personal communication, June 9, 1966.

## Description

*Camptotheca* is a rapidly growing deciduous tree (fig. 1), which reaches a maximum height of 25 meters (5, 11, 29). Its pale-gray rough bark is cracked and fissured. The trunk, which is up to 60 cm. in diameter, is commonly slender in relation to the height of the tree. The lower one-fourth to one-third of the trunk is often without branches.

The leaves (fig. 2) are simple, alternate, pinnate veined, ovate, acuminate, serrate or entire, and up to about 15 cm. long. The tree flowers in July and early August in China. The white polygamous flowers (fig. 3) are borne on the current season's growth in capitate inflorescences, which are solitary or arranged in simple panicles of three to six or more. Ten white exerted stamens are inserted on an epigynous, cup-shaped disk subtended by a five-toothed calyx. The ovary has one cell. The mature fruit (fig. 4) is an almost wingless samara about 2.5 cm. long with one seed. When dry it is shiny brown and has a leathery surface.

According to Tang (19), the wood is light brown, sometimes with dark-tinged sapwood. Otherwise demarcation between heartwood and sapwood is indistinct. The wood is moderately to extremely soft with a rather curly grain, which is fine to very fine in texture. It will not take a smooth cut, but can be split off along the rings. Tangential fissures and radial cracks develop when the wood is air dried. Growth rings are moderately distinct and may be exceedingly wide. The wood has fine rays and small, numerous, evenly distributed pores.

Wood samples from California agree generally with Tang's description. They are a very pale yellow brown, almost ivory. Most specimens have darkened to a pale gray brown near the outer surface of the wood cylinder. This darkening is very irregular in depth, extending inward from a small fraction of a centimeter to 4 cm. Annual rings are wide only near the center of the tree. The size of the rings indicates that maximum increase in diameter comes usually in the second year. Most of the samples suggest that the tree reaches a diameter of 6 to 8 cm. by the end of the fifth year. The pith is rarely in the center and may be offset considerably. This pattern of unequal growth seems to occur throughout the life of the tree.

The somatic chromosome number is  $2n = 42$ .<sup>4</sup> This count was determined from young seedlings, which were the progeny of trees growing at the U.S. Plant Introduction Station, Chico, Calif.

## Distribution

The distribution of *C. acuminata* is shown in figure 5. This map is based on that of Eyde (8) and on records from herbarium specimens and reliable published reports (28).

This tree occurs in the Southeastern Provinces of China from

<sup>4</sup> Raven, P. H., Stanford University, Stanford, Calif., personal communication, Aug. 10, 1966.





BN-27957

FIGURE 1.—*Camptotheca acuminata* "near Shih-Fang (Shih-Tang) Hsien, China, height 80 ft., circumference 4 ft., altitude 2,000 ft.," photographed on May 19, 1908. Lower branches have probably been cut for firewood. (Photograph by E. H. Wilson, neg. No. 66; courtesy of Arnold Arboretum, Jamaica Plain, Mass.)



BN-35338

FIGURE 2.—Branch of *Camptotheca acuminata* with terminal mature inflorescence.

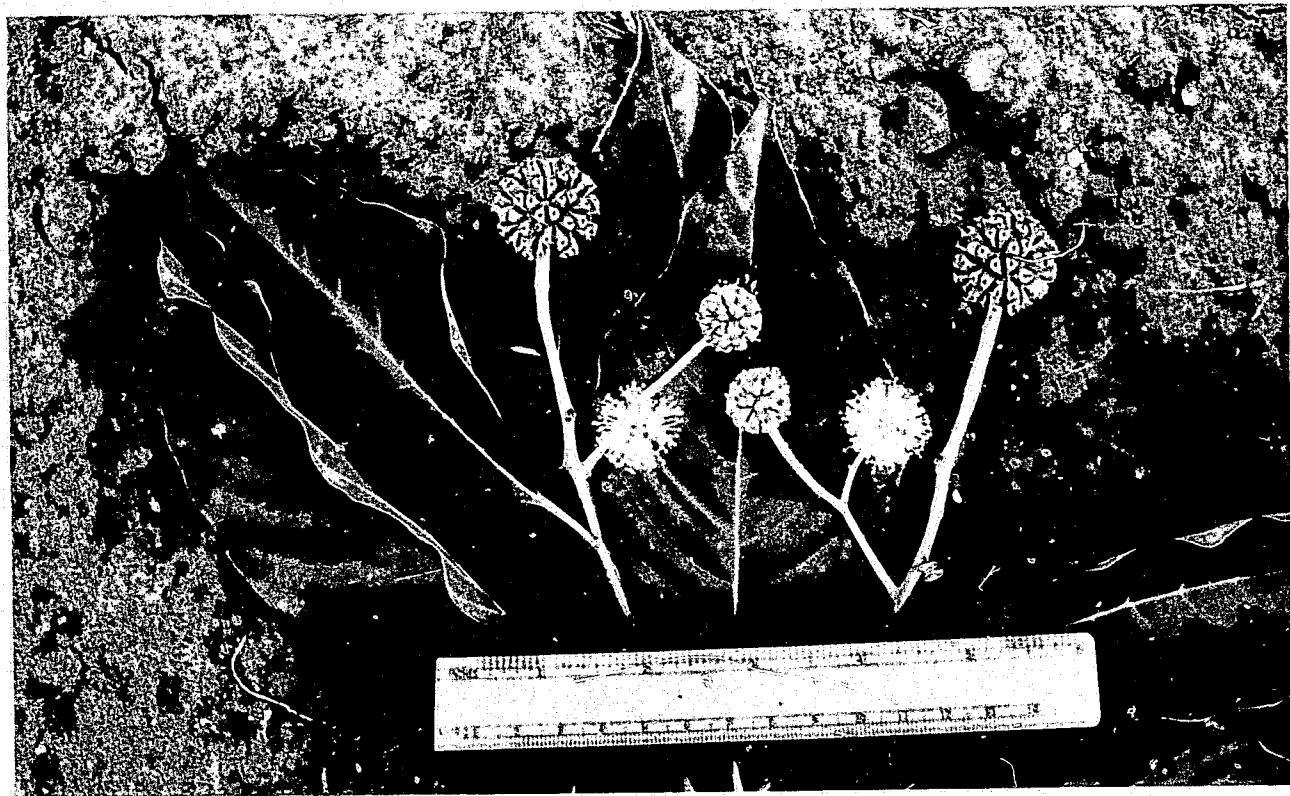
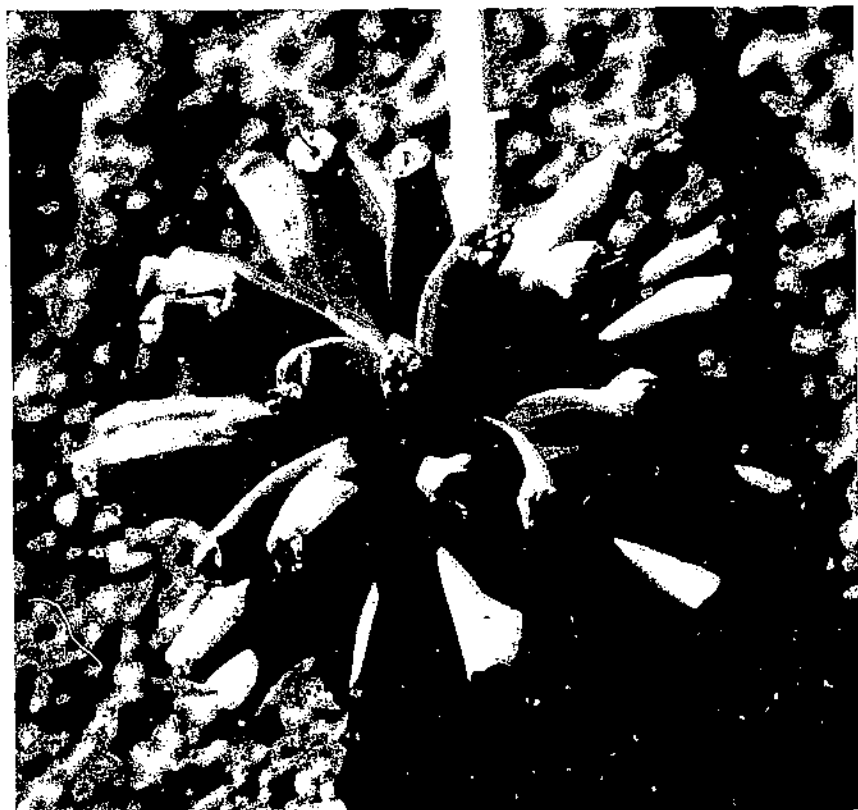


FIGURE 3.—Branch of *Camptotheca acuminata* with inflorescences in flower and early fruit.

BN-35839



BN-35340

FIGURE 4.—Mature inflorescence of *Camptotheca acuminata*.

Szechwan's Red Basin east to southern Anhwei and northern Chekiang and south to Yunnan, Kwangsi, and Kwangtung. It is not native to Hainan or Taiwan. The most southwesterly record in Yunnan is approximately 160 km. from the Burma frontier. The tree probably grows in Burma and possibly in northern Thailand.

Most collections of *Camptotheca* are from elevations of 300 to 1,100 meters. Occasional specimens were collected as low as 150 meters. In Yunnan the tree is recorded from as high as 2,400 meters.

### Environment

*C. acuminata* is a constituent of the Chinese mixed mesophytic forest formation (28). This formation includes many species, most of which are deciduous and none of which predominate. Most genera in the formation occur primarily in warm, moist, temperate regions.

Much of the natural mixed mesophytic forest area is intensively cultivated and forest occurs largely as remnants. On hills and

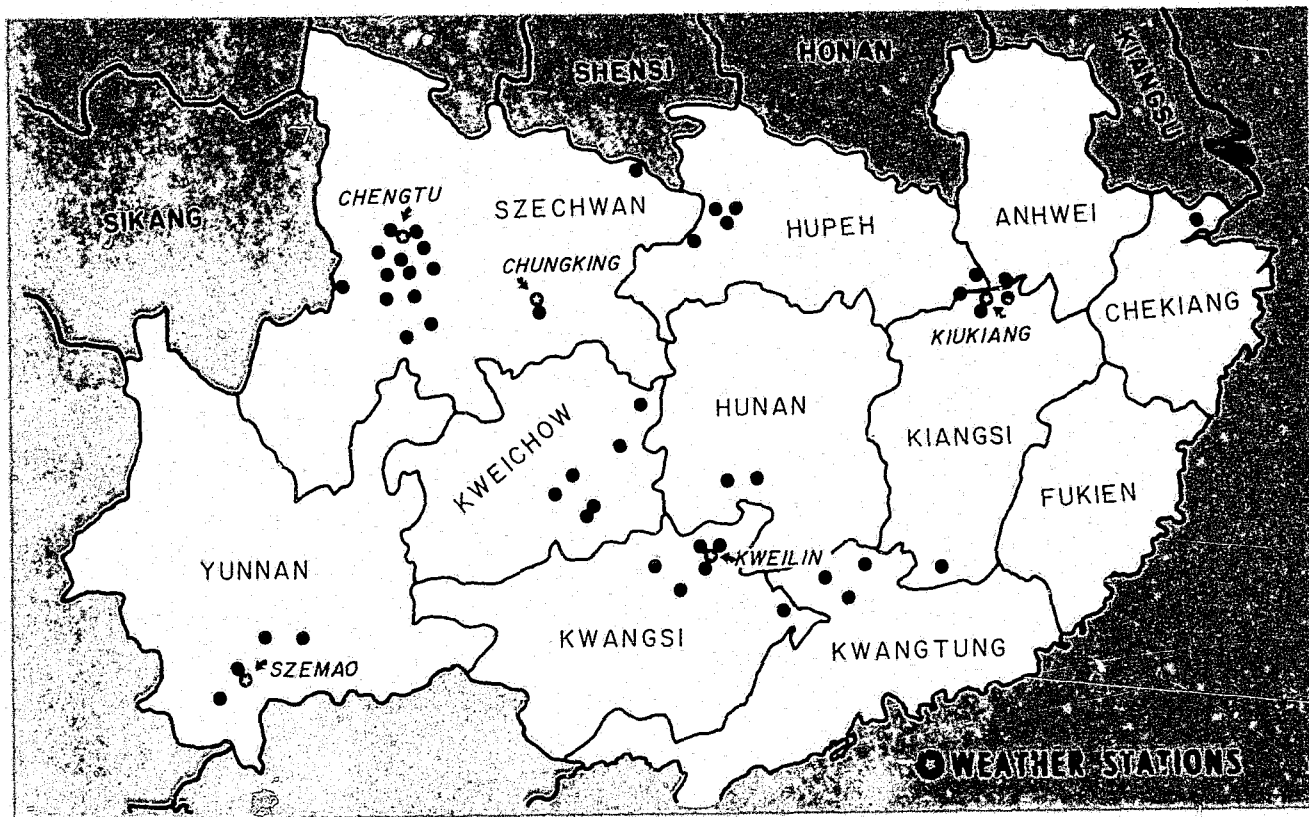


FIGURE 5.—Natural distribution of *Camptotheca acuminata* in southeastern China.

mountains this vegetation type occurs above broadleaf evergreen and below montane coniferous forests.

The northern boundary of the mixed mesophytic forest lies approximately along the Tsinling Mountains, which separate the watersheds of the Yellow and Yangtze Rivers. This east-west range is approximately at the 34th parallel and separates the wheat region of north China from the rice region of central and south China (14).

*Camptotheca* seems to be especially abundant in Szechwan's Red Basin. Wilson (30) commented that the tree is "particularly abundant by streams in the northern parts of the Chengtu Plain where it ascends to an altitude of 750 metres." The mild climate of this area is exemplified by the cultivation of rice as the principal summer crop with corn, sugarcane, tobacco, beans, potatoes, millet, and sorghum as important secondary crops. Wheat and rape are the principal winter crops. Tea and tung are important perennial crops (14).

Climatic data from five weather stations representative of the *Camptotheca* environment are shown in figure 6.<sup>5</sup> Herbarium records indicate that *Camptotheca* grows naturally at or very near each station (fig. 5).

Near Szemao (elevation 1,319 m.) it is recorded at 900, 1,800, and 2,400 meters. In Szechwan's Red Basin (Chengtu 498 and Chungking 261 m.) the tree is abundant at elevations of 450 to 1,100 meters. There are many herbarium records from Mt. Omei, 160 km. southwest of Chengtu, at 700 to 1,500 meters. Lü Shan, the type locality of this species, is but a few kilometers south of Kiukiang (elevation 32 m.). Here *Camptotheca* apparently grows at about 150 to 1,000 meters. Specimens are available from near Kweilin (167 m.) but do not include data on elevation.

At all stations January is the coldest month, with a mean minimum from 0.4° to 5.7° C. Mean January temperatures range from 3.4° to 11.2°, but four of the five stations have means of 6.2° or higher.

Precipitation in the *Camptotheca* environment ranges from about 100 to 200 cm. per year. Much of it falls during the peak of the summer growing season. During the October to March period, Chengtu, Chungking, and Kiukiang receive only 11, 23, and 34 percent, respectively, of the total rainfall recorded at these stations.

The growing season at these stations ranges from 272 to 344 days.

A unique characteristic of the *Camptotheca* environment is the unusually small amount of sunshine. The annual mean percent of possible sunshine ranges from 26 and 28 at Chengtu and Chungking to 41 and 46 at Kiukiang and Szemao, respectively. During June, July, and August the percent ranges from 23 at Szemao to 51 at Kiukiang. In comparison, the June-August percent of possible sunshine is 60 to 70 in the Southeastern United States east of the

<sup>5</sup> Climatic data provided by A. Yao, Environmental Data Research Laboratory, U.S. Weather Bureau.

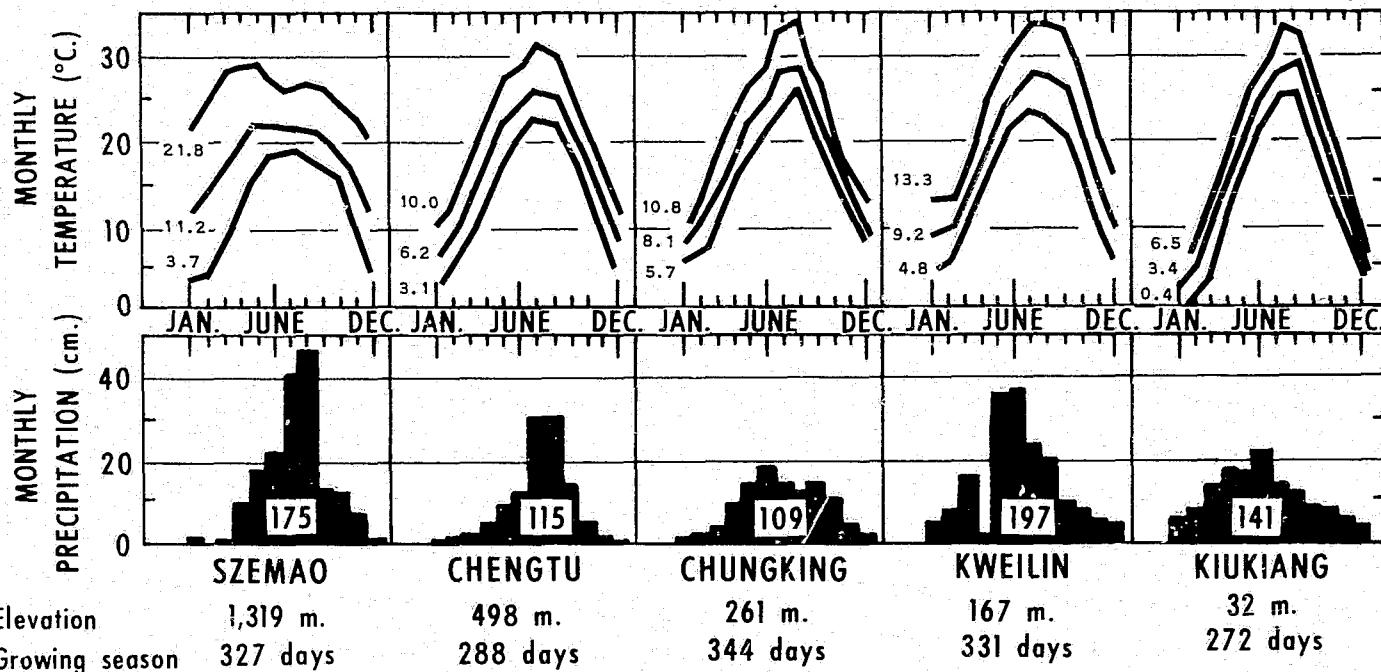


FIGURE 6.—Mean maximum, mean, and mean minimum monthly temperatures, precipitation, elevation, and length of growing season at five weather stations in southeastern China representative of *Camptotheca acuminata* environment. [Length (years) of temperature and precipitation records, respectively, at Szemao, 3 and 4; Chengtu, 21 and 21; Chungking, 28 and 60; Kweilin, 9 and 13; and Kiukiang, 14 and 52.]

Mississippi River and 70 to 90 over most of the Central and Western United States, decreasing abruptly to under 50 along a narrow coastal strip of the Pacific Northwest.

Relative humidity in the *Camptotheca* environment is high throughout the year. The annual mean at most stations is 80 to 83 percent. Monthly means do not fluctuate appreciably through the year. Extremes are about 75 and 87 percent.

*Camptotheca* is certainly more flexible in its environmental requirements than this summary of climatic factors suggests. Young plants survived for several years with minimum protection at Glenn Dale, Md., where the mean January temperature is 1.3° C. and the growing season is but 172 days. The two trees at Chico, Calif., have escaped winter damage, grown reasonably well, and produced good fruit crops. Here the mean January temperature is 7.3° and the minimum recorded is -11.7°. The growing season is only 234 days. Mean annual precipitation is about 60 cm., but the trees have been irrigated monthly during the dry summer season. In contrast, unirrigated trees at San Marino, Calif., with a mean annual precipitation of 50 cm., have grown satisfactorily for about 15 years, though they have not set good fruit. The growing season is about 315 days.

In a study of the ecological crop geography of China, Nuttonson (13) cited weather stations in the United States as climatic parallels of several Chinese stations representative of the *Camptotheca* environment. Szemao is regarded as analogous to Apalachicola, Fla., Chengtu to Frederick, Okla., Chungking to Ft. Worth, Tex., and Kiukiang to Tahlequah, Okla. No U.S. station is comparable to Kweilin. These comparisons are apparently based largely on mean minimum temperatures. Generally at the Chinese stations the rainfall is considerably greater and the growing season is much longer than at the analogous U.S. stations. Rainfall regimens at stations in both countries are essentially the same, with maximum precipitation during the growing season and a minimum in winter.

In China, *Camptotheca* apparently occurs most commonly on deep, well-drained, friable clay soils. These soils are similar to many of those in the piedmont and older areas of the Southeastern United States. Although they are slightly acid and of only medium fertility, they possess physical properties, such as granular surface layers and friable subsoils, that favor the growth of most plants. Most localities from which *Camptotheca* is recorded by herbarium specimens are in the red and yellow earth zones on the most recent soils map of China. These soils appear to be equivalent to the red and yellow Podzolic soils, according to the soil classification scheme in the U.S. Department of Agriculture Yearbook, "Soils and Men."<sup>6</sup>

*Camptotheca* is more likely to be found along moist valley bottoms than on the higher, drier slopes. Notes on a few herbarium specimens refer to *Camptotheca* sites as "dry" or "hillside," implying a well-drained location. Most of the notes indicate very

<sup>6</sup> This summary of soil conditions in the *Camptotheca* environment was provided by R. E. Zarza, Soil Conservation Service, U.S. Department of Agriculture.



moist locations, such as "swampy thicket," "bed of stream," and "side of stream."

### Relationships

The family Nyssaceae includes two other genera—*Davidia*, which is monotypic, and *Nyssa*, which has about seven species.

*Davidia involucreta* Baillon grows along the northern and north-western perimeter of the distribution area of *C. acuminata* (8). It occurs at elevations of 1,600 to 2,500 meters, well above the upper limit of *C. acuminata*.

*Nyssa* is largely a genus of eastern North America from Maine to Texas with outliers in southern Mexico. It includes two Asiatic species. Like *C. acuminata*, *N. sinensis* Oliver is a member of the Chinese mixed mesophytic forest. The geographic distribution of these two species is similar, but they apparently do not grow together. *N. sinensis* also occurs at elevations above the upper limit of *C. acuminata*.

*Nyssa javanica* (Blume) Wangerin grows in wet montane forests from Sikkim, Yunnan, and Kwangsi south to Java.

The wood anatomy of Nyssaceae was studied in detail by Titman (20). Eyde (8) reported on the structure of the fruit and the fossil record of the family. It is evident that *Camptotheca* is closely related to *Nyssa* and only remotely related to *Davidia*. Titman considered *Camptotheca* the most advanced genus of the family. Certain of its characteristics are intermediate between *N. sinensis* and *N. javanica*. In other characteristics it is more advanced than either. Titman suggested that *N. javanica* and *C. acuminata* shared a common ancestor, which evolved from *N. sinensis*. Eyde, taking into account Titman's work and his own studies of the genus, considered the derivation of *Camptotheca* from a *N. javanica* ancestor "a reasonable deduction." He further concluded from the fossil record and fruit morphology that *N. javanica* is closely related to *N. aquatica* L. of the Eastern United States.

### Uses

*C. acuminata* is used in China as an ornamental (18). It has been planted extensively in the Provinces south of the Yangtze River as a roadside tree.<sup>7</sup> It is widely planted along irrigation ditches in China and its branches are used for firewood (8). Farmers in Omei, Szechwan Province, plant the tree around farmhouses and near ricefields. They hang bundles of rice on its lower branches to dry.<sup>7</sup>

A note on an herbarium specimen<sup>8</sup> indicates this is a "drug plant" but provides no further information on this use.

<sup>7</sup> See footnote 3, p. 2.

<sup>8</sup> Keung Tse Paai, South China, July 22, 1921, (F. A. McClure, No. 6546); in Arnóld Arboretum herbarium, Jamaica Plain, Mass.

### Introduction of *Camptotheca* Into the United States

*Camptotheca* was introduced from China into the United States five times:

1911.—Seeds collected by E. H. Wilson (No. 4405) at Mt. Omei, Szechwan Province, were sown at the Arnold Arboretum in February 1911. Plants were supplied to the U.S. Department of Agriculture in November 1912 and accessioned as P.I. (Plant Inventory) 34534 (21). No records are now available indicating further distribution by either the Arnold Arboretum or the Department.

1927.—Plants collected in China by W. T. Swingle (No. 803) were received by the Department in January 1927 and accessioned as P.I. 71179 (22). The Department's published inventory does not record a more specific origin for this accession. Swingle's field notebooks are stored in the library of the University of Miami (Fla.). His notes are minimal and his handwriting hardly legible. There is no record of the origin of his No. 803. His notation is limited to "*Camptotheca acuminata* Nyssaceae flowers white clusters. Good street tree." Entries preceding and following his record for *C. acuminata* indicate the collection was made between December 2 and 4, 1926. According to Swingle's notebook and the Department's published inventory records, other plants he collected in early December 1926 came from Chekiang Province or Nanking.

From several circumstances associated with the shipment of 81 Swingle collections received in Washington, D.C., on January 12, 1927, it is reasonable to conclude that his *Camptotheca* was collected in eastern China, probably in the Nanking Botanical Garden. These circumstances include the nature and recorded origin of his collections, the relatively short period (about 5 weeks) between his collection of *Camptotheca* and its receipt in Washington, and knowledge that his principal mission was not the collection of plants.<sup>9</sup> According to Reidel (17), this introduction was available in Los Angeles in 1928.

1934.—Unidentified seeds were received by the Department in April 1934 and accessioned in April 1939 as P.I. 132293 (23). They were forwarded by A. N. Steward, College of Agriculture and Forestry, Nanking University, Kiangsu Province. They were collected by Steward (No. 75) "on a rocky slope at 600 feet altitude in Chang An, Yung Hsien." Seeds were sown at the Glenn Dale, Md., Plant Introduction Station in June 1934. Station records indicate 150 plants were available in August 1935. In April 1937, plants were distributed to the Atkins Garden near Cienfuegos, Cuba; the Honolulu Experiment Station of the Hawaiian Sugar Planters Association; Balboa Park in San Diego, Calif.;

<sup>9</sup> Although Swingle was employed by the Department during 1926-27, he was traveling under the auspices of, and with financial support from, the Library of Congress. He was deeply interested in oriental literature and was very active in building up the orientalia collection of the Library.

Federal Experiment Station, Mayagüez, Puerto Rico; and in May 1939 to the U.S. Plant Introduction Station, Chico, Calif. They survived only at Chico.

1935.—The Arnold Arboretum received seeds from the Lü Shan Arboretum and Botanical Garden at Kuling near Kiukiang (Arboretum Introduction No. 128-35). They were sown on February 26 and germinated 3 weeks later. Plants were potted for the arboretum collection but were recorded as dead in 1942. Apparently this introduction was not distributed.<sup>10</sup> Reidel (17) indicated that a few plants were received in Los Angeles in 1941, but they did not survive shipping. This may refer to the 1934 or 1935 introduction.

1949.—Willard Hagen, nurseryman in Arcadia, Calif., obtained seeds from the Lü Shan Arboretum and Botanical Garden.<sup>11</sup> Plants raised from these seeds were distributed to private purchasers, city parks, and botanical gardens on the west coast from California to Washington.

### History of *Camptotheca* in Antitumor Screening Program

Had the identity of A. N. Steward's seeds been known at the time of their receipt, it is unlikely they would have been planted. Plants were available in the California nursery trade but had not met with favor. However, Steward's seeds were planted so as to have specimens for identification and to provide living specimens for evaluation if this later proved desirable. Two *Camptotheca* trees from his seeds are now growing at the U.S. Plant Introduction Station in Chico, Calif.

In 1950, scientists in the U.S. Department of Agriculture began a search for sources of steroidal sapogenins that could be converted into cortisone. In initiating such a program, chemical laboratories can be equipped rapidly, but plant materials can be procured only as nature permits and field projects can be planned. To avoid delay in getting the cortisone program underway, botanists turned first to their most accessible sources. Included were plant introduction stations in California, Florida, Georgia, and Maryland, which maintain, in permanent plantings, hundreds of species from over 300,000 plant introductions brought into the United States since 1898. Leaves of *Camptotheca acuminata* were among the plant samples provided by the Chico station. An extract prepared in the Department's Eastern Utilization Research and Development Division (EURDD), Wyndmoor, Pa., gave positive tests for flavonoids, tannins, and sterols, but negative tests for sapogenins and alkaloids (24).

Extracts of plants eliminated by screening as potential sources of cortisone precursors accumulated at EURDD. This collection of extracts, many of plants unlikely to be readily available again,

<sup>10</sup> Schubert, B. G., Arnold Arboretum, Jamaica Plain, Mass., personal communication, June 3, 1966.

<sup>11</sup> Hagen, Willard, personal communication, May 29, 1966.

provided an excellent basis for further screening. J. J. Willaman, then Chief of the Biochemical Section, and M. E. Wall, then Head of Plant Steroid Investigations, EURDD, arranged for further testing of some of these extracts for antibiotic, antiviral, and antitumor activity. In 1957, 1,000 extracts, including *Camptotheca*, were forwarded to the Cancer Chemotherapy National Service Center for antitumor screening. The extract of *Camptotheca* leaves was active in two tumor systems, but the supply was inadequate to complete the required series of tests.

In 1960 Wall joined the Research Triangle Institute, Durham, N.C., to head up the RTI program established under a contract with the CCNSC. He reviewed antitumor screening data on the old extracts and requested new supplies of *Camptotheca* and other plants that had shown preliminary activity.

The Chico station provided new samples of *Camptotheca* leaves, twigs, and fruit in September 1961. Leaves were inactive but fruit and twigs were active against lymphoid leukemia L-1210. The activity of these *Camptotheca* extracts was accompanied by lack of toxicity to the test animals. In 10 tests with extracts of the fruit, involving 60 animals at dose levels from 44 to 800 mg. per kilogram of body weight, all animals survived.

To assure a supply of material so as to continue chemical research on isolation and identification of the active constituent, a large limb was removed from one of the Chico trees and separated into individual samples of bark, bark-free wood, and twigs. Twigs were inactive, but bark and wood showed good activity.

By late 1963 intensive chemical research was underway at the Research Triangle Institute to isolate and identify the substance responsible for *Camptotheca's* antitumor activity. It was evident that the two trees at Chico would not provide adequate raw material to complete the critical research still necessary unless both were completely removed. The interior of mainland China was off limits to American botanists and consequently could not be considered a source of supply. As far as was known at that time, the Chico trees were the only living specimens in the United States and possibly the only specimens available outside of mainland China. It was obviously unwise to destroy this critical source, for it could prove to be the only supply of propagating material.

In late 1963 a systematic survey was undertaken of west coast locations that might have one or more living specimens. Contacts included staff members at colleges and universities, botanical gardens, nurseries, and city parks. Thirteen trees were located in central and southern California. Arrangements were made to uproot and remove most of these trees as additional research material was needed.

Horticulturists at the Chico Plant Introduction Station succeeded in germinating seeds from the 1963 crop. A representative sample of seedlings collected in September 1964 was active. The active constituent seemed not to be present in as great abundance as in more mature growth, but it was available in workable quantity. This finding and other tests indicated that the active constituent seemed to be present in most parts of the plant, at all

stages of growth, and during all seasons of the year. Future supply was reasonably certain.

During the late summer of 1965, many of the California trees were harvested. The supply of raw material then on hand was regarded as an adequate source of camptothecin for all research leading up to and including preliminary clinical tests. However, the yield of camptothecin from the first large-scale isolation did not meet estimates.

After further inquiry in 1966, eight additional trees were located in a Taiwan botanical garden. An appeal through a nurseryman's trade magazine was rewarded with information on two additional trees in California. In 1967 four of the Taiwan trees and the two newly discovered California trees were removed as an additional source of raw material. The seedlings remaining from the original planting at Chico were also harvested.

A new planting of 1,300 seedlings was established at Chico during the spring of 1966 and a third planting of 5,000 seedlings in 1967. They will be available for harvest at the end of their second growing season.

### *Camptotheca* Specimens Used in Antitumor Screening Program

Information about individual trees used in the antitumor screening program is given in table 1.

The origin of few of these *Camptotheca* trees can be determined precisely. However, if it is assumed there were no introductions other than those of which we are aware, the age of a tree is useful in identifying it with a specific introduction. Age can be misleading, whether based on records or a count of annual rings. Records may indicate when a tree was set in its permanent location, not necessarily when seeds were germinated. Annual-ring counts are not likely to accurately reflect the number of years a seedling may have remained in a greenhouse before being planted in its permanent site.

The tree from the University of California Botanical Garden originated as a seedling from a tree in a private garden near the university. The parent specimen was destroyed about 1954. Observers recall that it was 6 to 8 meters tall and the trunk diameter was 25 to 30 cm. These dimensions suggest an age of 30 years or more. This tree may be a descendant of the 1927 introduction, but it seems more likely to be a descendant of P.I. 34534 introduced in 1911.

The specimen from a private garden in Los Angeles and the Oakland tree are probably from the Hagen introduction. This assumption is suggested by their age and the fairly wide distribution of the Hagen introduction to private purchasers, city parks, and botanical gardens. Specimens from the Hagen introduction were also distributed to the Saratoga Horticultural Foundation in California and the University of Washington Arboretum at Seattle. The Saratoga specimens were discarded when the plants

TABLE 1.—Data on *Camptotheca* trees used in antitumor screening program

Location	Trees	Trunk diameter		Age (years) and basis for determination		Origin	Remarks
		Height	Meters	Number	Records		
Arcadia, Calif. (Los Angeles State and County Arboretum).	{	---	---	---	About 15 in 1967 (records).	Hagen introduction	Received from Hagen Nursery in 1952.
						Cutting from above	Both remain in Los Angeles State and County Arboretum.
Chico, Calif. (U.S. Plant Introduction Station).	2	9.0	15	33	in 1967 (records).	P.I. 132293	Planted at Chico in 1939; both remain at Chico.
Kensington, Calif. (Blake Garden, University of California).	1	11.0	18	About 25 in 1965 (estimated).		Unknown	Removed for present program in 1965.
Los Angeles, Calif. (University of Calif. Botanical Garden).	1	6.0	10	About 13 in 1965 (records).		Probably P.I. 34534	Do.
Los Angeles, Calif. (private garden).	1	6.0	15	15 in 1967 (annual rings).		Probably Hagen introduction.	Removed for present program in 1967.
Oakland, Calif. (Oakland Park Department).	1	8.5	27	16 in 1967 (annual rings).		do	Do.
San Marino, Calif. (Huntington Botanical Garden).	{	18.0	35	31 in 1965 (annual rings).		Probably P.I. 71179	Removed for present program in 1965.
				7	4.5-6		
Taipei, Taiwan (Botanical Garden, Taiwan Forest Research Institute).	8	6.0	15	19 in 1967 (records).		Introduced from National University, Hengchow, China.	4 removed for present program in 1967, 4 remain.
Whittier, Calif. (city park).	1	7.5	20	Unknown		Hagen introduction	Remains in Whittier city park.

proved of no ornamental value. The Washington planting was killed by a severe early freeze in 1955.

The age of the original Huntington Botanical Garden tree suggests that it is a descendant of the 1934 introduction. However, records of the distribution of this introduction are still available and do not indicate that Huntington received plants. More important, the first distribution of this introduction was not until 1937. In 1965, when this tree was removed, a cross section of the trunk showed 31 distinct annual rings and the tree must have been in place at least since 1934. It is probably a descendant of P.I. 71179, though it could have originated as a cutting or seedling from the 1911 introduction. It was the parent of seven trees at the Huntington Botanical Garden, one of which is shown in figure 7.

### Antitumor Activity of *Camptotheca* and Related Genera

Aqueous and ethanolic extracts of most samples of Nyssaceae were tested in the routine CCNSC screening program against lymphoid leukemia L-1210, adenocarcinoma 755 (Ca-755), and sarcoma 180 (Sa-180). Some were also screened against other tumor systems, such as Lewis lung carcinoma (LL) and the human sarcoma in the embryonated egg test system (HS-1). A few samples were submitted after a change in procedure, which provided for screening a single aqueous ethanolic extract of each sample.

Screening procedures for all tumor systems are described in the CCNSC protocols (4) and unpublished supplements to these protocols available from the CCNSC. They are summarized in screening reports of the CCNSC (1, 2, 3). For an extract to demonstrate "confirmed" activity, it must significantly reduce tumor growth in a series of four bioassays. Extracts that pass two single dose tests are assayed at three or four dose levels in a third and fourth test. To pass these last two tests, or "dose-response experiments," an extract must significantly reduce tumor growth at a dose level at which animal weight loss is within acceptable limits (4). Excessive weight loss indicates toxicity.

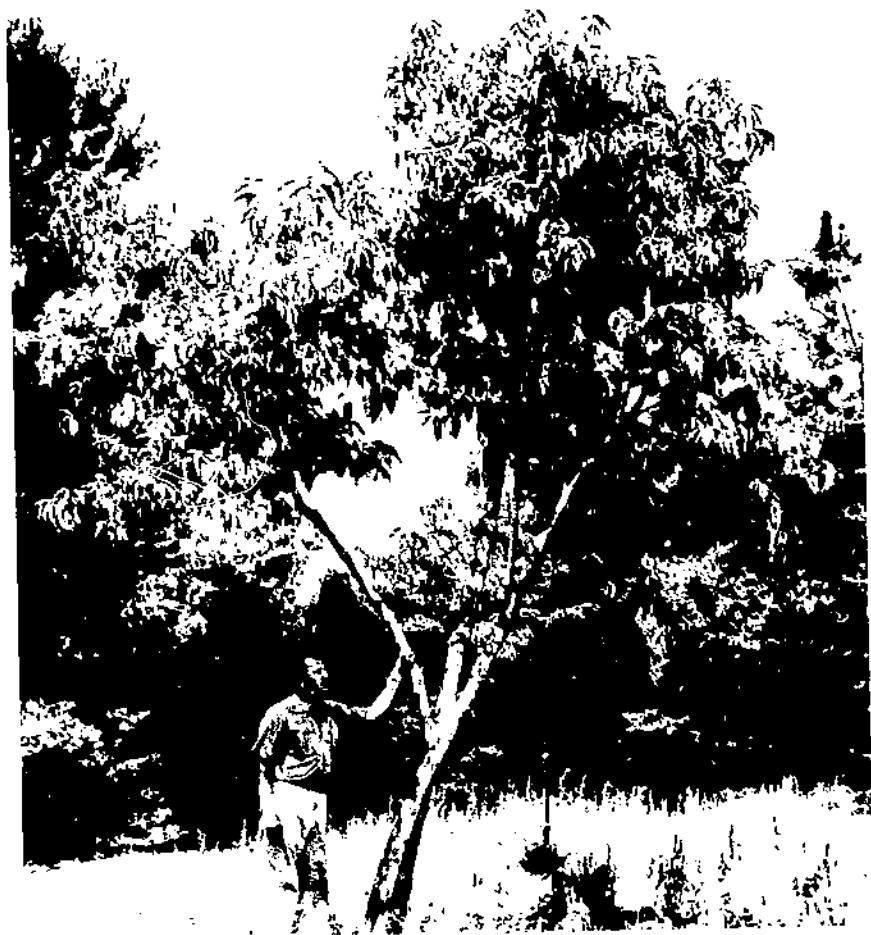
The activity of crude *Camptotheca acuminata* ethanolic extracts against L-1210 was reported in detail by Perdue and others (15). The results were based on the bioassay of 106 samples. Nineteen specimen trees from 13 to 35 years old were represented by 98 samples taken at eight locations. Seven of these trees were at one location. Seedlings 1 and 2 years old were represented by eight samples.

Seventy percent of the leaf extracts were negative in tests against L-1210, but most extracts of other parts of the tree were active. The greatest L-1210 inhibition was shown by root extracts. Extracts of intact roots (with bark), root bark, and bark-free root wood were generally superior to extracts of all above-ground parts of the tree. Extracts of bark-free stem wood were more active than those of other aboveground parts. Extracts of older twigs one-fourth to 1 inch in diameter were considerably

more active than those of younger twigs one-fourth inch or less in diameter.

The season of collection appeared to have no effect on activity. Material harvested at any time of year can be expected to show activity when tested against L-1210. The degree of activity did not appear to be related to the age of the trees from which samples were collected.

The activity of samples was assumed to be directly related to their camptothecin content. The bioassays suggested that the maxi-



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FIGURE 7.—*Camptotheca acuminata* at Huntington Botanical Garden, San Marino, Calif., at 14 years of age in August 1964. This tree has not been irrigated. Annual mean precipitation here is about 50 cm. The shape of the tree is not typical of this species. The lower branches were removed as raw material for chemical research to isolate and characterize the active constituent.



imum yield of camptothecin would be obtained by harvesting trees at the stage when they produce maximum yields of dry matter.

An extract of the first *Camptotheca* leaf sample tested in this program showed preliminary activity against Ca-755 as well as L-1210. The extract of fruit that first showed confirmed activity against L-1210 also showed sufficient activity against HS-1 to meet CCNSC criteria for confirmation.

Pure camptothecin displayed high activity against the solid tumor Walker 256 Intramuscular (WM) in rats. Doses as low as 1.25 mg. per kilogram of body weight significantly inhibited tumor growth (27). Like L-1210, the WM tumor system is highly predictive for clinical activity in man (9).

Pure camptothecin is also moderately cytotoxic against cells derived in culture from a carcinoma of the human nasopharynx, cell line KB (Eagle). The effective cytotoxic dose (ED<sub>50</sub>) is 0.74 µg. of camptothecin per milliliter of solvent (27). This dose inhibits growth of KB cells in test tube culture to 50 percent of growth of cells in similar but untreated cultures.

Other species of Nyssaceae included in the antitumor screening program are as follows:

Species	Samples tested
<i>Davidia involucrata</i> Baillon	Fruit (two samples).
<i>Nyssa aquatica</i> L	Twigs and leaves (two samples).
<i>N. biflora</i> Walter	Twigs and leaves; twigs, leaves, flowers.
<i>N. javanica</i> (Blume) Wangerin	Twigs, leaves, stem bark.
<i>N. ogeche</i> Marshall	Fruit; twigs, leaves, fruit.
<i>N. sylvatica</i> Marshall	Twigs and leaves (five samples); stem bark (five samples); wood with bark intact; roots with bark intact; fruit (two samples).

No antitumor activity of consequence was demonstrated by any extract of *Davidia*, *Nyssa aquatica*, *N. biflora*, *N. javanica*, or *N. ogeche*.

Fourteen samples of *N. sylvatica* were screened (10 against Sa-180), and at least minimal activity was shown by extracts of seven samples. With one exception, the activity of these extracts was against Sa-180. Most extracts were negative against all other tumor systems.

Extracts of four samples of *N. sylvatica* showed more than minimal activity. An aqueous extract of a sample of stem bark was active against LL. An aqueous extract of a second sample of stem bark and ethanolic extracts of two samples of twigs and leaves were active against Sa-180. Each extract of stem bark passed two preliminary tests but failed confirmation in subsequent dose-response experiments. The two extracts of twigs and leaves significantly reduced tumor growth in two dose-response experiments. However, excessive weight loss by the test animals indicated that the reduction in tumor growth was due to toxicity and not to antitumor activity. Almost all positive tests with extracts of *N. sylvatica* samples were marginal.

This review on the performance of *Davidia* and *Nyssa* extracts is based on published (1, 2, 3) and unpublished screening data from the CCNSC.

## Chemical Analysis of Fruit

Fruit from each of the two *Camptotheca* trees at Chico, Calif., was routinely tested in the oilseed-screening program of the U.S. Department of Agriculture. Preliminary analyses are designed to detect seed oils that are different in composition from the usual vegetable oils, like those of soybeans, corn, cotton, linseed, and peanuts.

Details on the analytical procedures used are given in the early reports on the oilseed-screening program (7, 12).

The fruit from tree K5-6 weighed 38.4 gm. per 1,000 and had 12.4 percent protein and 14 percent oil. Fruit from tree K6-6 weighed 31.6 gm. per 1,000 and had 12.3 percent protein and 16 percent oil. Neither sample contained starch. Supplementary tests (thin-layer chromatography, infrared analysis, and ultraviolet analysis) indicated that unusual substances were not present in any appreciable amount. The oil was semisolid and sticky and obviously contained some minor constituent not present in the usual oils.<sup>12</sup>

## Culture

Plantings of *Camptotheca* at the Chico Plant Introduction Station (fig. 8) were primarily designed to provide raw material to assure adequate supplies of camptothecin for biological, chemical, and clinical research.

Some preliminary data on the culture and performance of these plantings are included, because such information is meager and the data lend reasonable support to a tentative conclusion that intensive agricultural production of *Camptotheca* is possible.

## Fruit Yield and Germination

The two *Camptotheca* trees at Chico regularly produce a fruit crop. Mature inflorescences were collected from each tree on November 16, 1965. Yields were 9.54 and 13.90 pounds, drying to 3.45 and 4.96 pounds, respectively.

The good fruits per inflorescence from the higher yielding tree averaged 47. There were approximately 9,700 good fruits per pound of dry mature inflorescences. The number of dry fruits per pound was 11,325.

Fruit harvested in November 1964 and held at 4.4° C. deteriorated in storage. Less than 1 percent of the fruit germinated in the spring of 1965. Germination tests with seed and fruit from the 1963 and 1965 crops are shown in tables 2 and 3. Seed planted in the fall immediately after harvest gave 76 percent germination if removed from the fruit, but only 2 percent if allowed to remain in the fruit (table 2).

<sup>12</sup> Unpublished data provided by F. R. Earle, Agricultural Research Service, U.S. Department of Agriculture.



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FIGURE 8. *Casipouita monacata* at U.S. Plant Introduction Station, Los Angeles, Calif. This seedling, part of planting started in greenhouse in late 1963 and transplanted to field in spring of 1964, was 2.4 meters high in early April 1966 as shown here.

Germination is apparently hindered by excessive drying at elevated temperatures. Fruit dried at 48.9° C. for 12 hours and 3 or 65.6° C. for an additional 12 hours gave only 20 percent germination. In contrast, reasonably satisfactory germination was achieved both with fruit dried for a shorter period (4 hours) at high temperature (65.6°) (table 2) and with fruit dried for a

TABLE 2.—*Germination tests with seed and fruit from 2 Camptotheca acuminata trees at Chico, Calif., 1968<sup>1</sup>*

Treatment (° C.)	Seed or fruit	Date planted	Approximate germination time	Germination
	Number		Days	Percent
Seed removed from fruit . . . . .	50	Nov. 13	21	76
Fruit planted direct . . . . .	50	do.	(?)	2
Fruit dried 4 hours at 65.6° . . . . .	100	do.	27	68
Fruit dried 12 hours at 48.9° plus 12 hours at 65.6° . . . . .	120	Nov. 18	26	20
Fruit stratified in moist sand at 4.4° Nov. 13–Dec. 16: . . . . .				
Undried . . . . .	90	Dec. 16	19	75
Dried 4 hours at 65.6° . . . . .	200	do	19	94

<sup>1</sup> Combined sample from trees K5-6 and K6-6, harvested Nov. 13, 1963, and planted in greenhouse flats.

long period (12 hours) at lower temperature (46.1°) (table 3). Satisfactory germination was also attained when the fruit was stratified in moist sand for approximately a month regardless of whether or not it was dried after harvest (table 2).

The 1965 fruit crops from trees K5-6 and K6-6 were tested separately, and stratification for 30 days was compared with a similar period of storage at room temperature (table 3). Reasonably satisfactory germination was achieved with all four samples. Stratified fruit gave slightly better germination than unstratified fruit.

Fruit germination should not be a limiting factor in growing *Camptotheca*. Germination can be increased either by moderate drying with artificial heat or by stratification. The most satisfactory treatment will probably be a combination of these two procedures.

TABLE 3.—*Germination tests with fruit from 2 Camptotheca acuminata trees at Chico, Calif., 1966<sup>1</sup>*

Tree No. and treatment (30 days)	Fruit	Approximate germination time	Germination
	Number	Days	Percent
K5-6:			
Stratification, moist sand	1,000	14	83.4
Stored at room temperature	100	14-21	78.0
K6-6:			
Stratification, moist sand	1,000	14	66.8
Stored at room temperature	100	14-21	69.0

<sup>1</sup> Fruit harvested Nov. 16, 1965, dried 12 hours at 46.1° C., stored dry in polyethylene bags at 4.4° until Dec. 21, 1965, and planted Jan. 20, 1966, in greenhouse flats.

### Fertilizer Tests

Seedlings of *C. acuminata* established from the 1963 fruit crop were used in limited fertilization tests. A single row of seedlings was selected and treated as a randomized complete block design of three blocks with three treatments each and five trees per treatment.

Ammonium sulfate was applied on July 16, 1965, at 500 and 1,000 pounds per acre to appropriate plots in each block. A third plot in each block was not treated with fertilizer. At the beginning of this test, mean trunk diameters were 2.2, 2.3, and 2.6 cm. in plots with 0, 500, and 1,000 pounds of fertilizer per acre, respectively. Respective increases in trunk diameter 8½ months later on March 30, 1966, were 0.46, 0.56, and 0.91 cm.

There was a trend toward an increase in mean trunk diameter with increasing rate of fertilizer application. These differences were not significant at the 5-percent level. However, time did not permit extending the test through the spring and early summer of 1966, when maximum growth would be anticipated.

On June 25, 1966, plants from these test plots were harvested for preparation of extracts and biological evaluation. Mean dry weights per plant were 1.4, 2.3, and 2.9 pounds for plants from plots treated at 0, 500, and 1,000 pounds of fertilizer per acre, respectively.

### Tree Growth and Yield

Spring growth begins about the last week in March. Inflorescences start developing in late May and by mid-June are about 0.6 cm. in diameter. Trees are usually in about 50 percent of full flower by mid-August. Fruits are fully mature by mid-November. Growth continues until the first frost, which was November 14 in 1964 and November 28 in 1965.

Many botanists have noted the rapid growth of *Camptotheca*. The initial planting of seeds at Chico was started between November 13 and December 16, 1963. By late January seedlings were 5 cm. high. They were transplanted to pots and then to the field after the danger of frost was past. They were spaced at intervals of 45 cm. in rows 1.2 meters apart at approximately 7,260 plants per acre.

In late August 1964 the seedlings averaged about 45 cm. high with woody stems 1.2 cm. in diameter. In 1965 their mean height increased to about 1.4 meters by mid-June and 2 meters by early October.

In 1966 the larger seedlings in late June were 2.7 meters tall, with a mean of 2.3 meters. The mean trunk diameter was 4 cm. Three of these seedlings were forming flower heads. The larger seedlings by late August were 4.3 meters high.

Average yields harvested in late June were leaves 3 and stems 3.4 pounds (fresh weight) per seedling. These samples dried to 1 and 1.5 pounds, respectively. In another group of seedlings mean yield of roots was approximately 0.6 pound (dry weight) per plant.

Yields of nonleafy raw material of about 7.5 tons (dry weight) per acre can be anticipated. Competition among individual plants in large plantings will undoubtedly reduce this yield. On the other hand, selection for higher yielding varieties can be expected to maintain or increase this yield.

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