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A SCANNING ELECTRON MICROSCOPY STUDY OF TAXUS LEAVES AS RELATED TO TAXONOMY

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Abstract

Scanning electron microscopy, when applied to the surfaces of the needles of *Taxus* spp. (yew) revealed features that appear useful in the taxonomy of this tree species which yields the important anticancer drug, taxol. For instance, all of the four North American species have 3-5 rows of stomata on one-half of the abaxial leaf surface, whereas all of the others, including those from Europe and Asia, have 7-10 rows of stomata. The appearance of individual or fused papilliform epidermal cells and their arrangement on the leaf surface also is a feature that varies between species. Patterns of wax formation appeared on all species of yew examined but none could characteristically be assigned to a given species. Wax pattern variation was dependent upon age, environment, and probably to some extent, species differences. This study may provide some additional useful and reliable indicators in *Taxus* taxonomy.

Key Words: Scanning electron microscopy, yew leaves, yew taxonomy, taxol, epicuticular wax, yew species, yew geography.

Introduction

The yew (*Taxus* spp.) is an evergreen in the order Taxales, and the family Taxaceae having between six to 10 or 11 species distributed throughout the north temperate zones of Asia, Asia Minor, India, Europe, North Africa, and North America (Hartzell, 1991). *Taxus* is thought to have descended from *Paleotaxus rediviva* which is known from the Triassic age fossil record. Recently, with the discovery that all *Taxus* species make the extremely important anticancer drug, taxol, interest in all aspects of the biology of this tree species have intensified. However, one biological aspect that has been relatively ignored is the need for more taxonomic descriptors that can be used to aid *Taxus* species identification. Problems are known to exist with yew taxonomy. It seems that species designation has often been based on geographical location rather than on morphologies. This has been true because of the relative lack of morphological and developmental indicators of this genus. We have examined the leaf surface structures using scanning electron microscopy (SEM) to provide potential new descriptors that may be useful in the taxonomy of yew and its relatives. For this purpose, we have conducted a SEM study to determine if various aspects of leaf morphologies can be correlated with some of the species descriptions that currently exist.

Hartzell (1991) pointed out that there may be ten species in the genus *Taxus* which include *T. baccata*, *T. sumatrana*, *T. floridana*, *T. brevifolia*, *T. chinensis*, *T. canadensis*, *T. wallichiana*, *T. cuspidata*, *T. globosa*, and *T. mairei*. He pointed out that these taxa are closely enough related to be considered by some to be intraspecific variants of a single species called *T. baccata*, which is native to Europe and the Mediterranean region.

The largest remaining natural population of yew is *T. brevifolia* which grows adjacent to the coast in northwestern United States and along the southwestern coast of Canada (Hartzell, 1991). Other species found in North America are *T. canadensis*, which forms a characteristically dense understory, is normally not more than six feet in height, and grows in northeastern United

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States and southeastern Canada, *T. floridana*, which grows east of the Apalachicola River in northwestern Florida, and *T. globosa*, which grows from the state of Neuvo Leon in northeastern Mexico to Guatemala and El Salvador. The most well-known species of yew is *T. baccata* which grows to an average height of 30 feet, is found in the British Isles and Scandinavia, and ranges from the Alps to the Baltic countries and East into the Mediterranean countries and North Africa. The species which is easiest to cultivate and which grows the fastest is the Japanese yew *T. cuspidata*. It is found on the islands of Hokkaido, Honshu, Shikoku and Kyusu and in Manchuria, eastern Russia, and in central Korea. This species commonly has a pyramidal or upright shape.

There is apparently still some confusion concerning the Asiatic yews. Hartzell (1991) referred to *T. mairei* as the species which grows in China and Taiwan. It grows up to 50 feet in height and may attain a circumference of 9 feet. However, a number of the asiatic yews may be the cultivars of the Asian yew *T. sumatrana*, which grows in India, Burma, China, Vietnam, Indonesia, Sumatra, Celebes, Philippines and Taiwan between 1,300 and 7,000 feet (ca. 400-2133 M). Another asiatic yew species is the Himalayan yew, *T. wallichiana*, which grows in Afghanistan, Burma, Bhutan, China, India, Nepal, Philippines and Tibet between 5,000 and 10,000 feet (ca. 1524-3048 M).

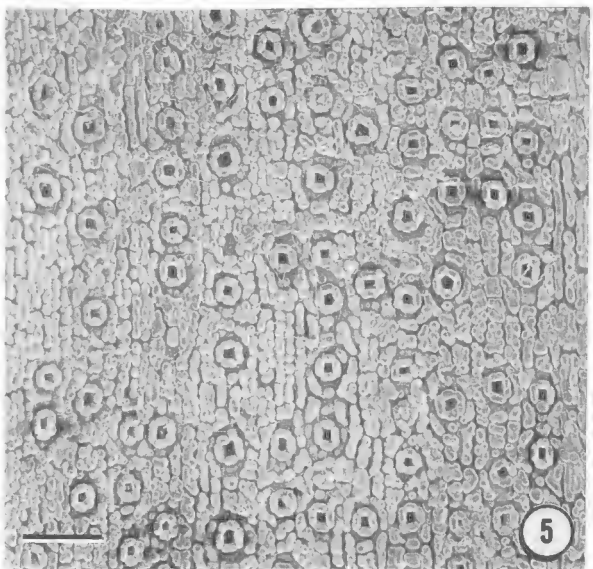
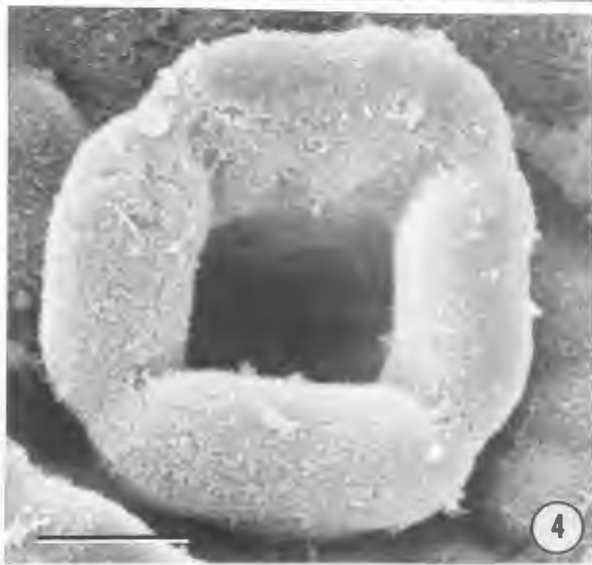
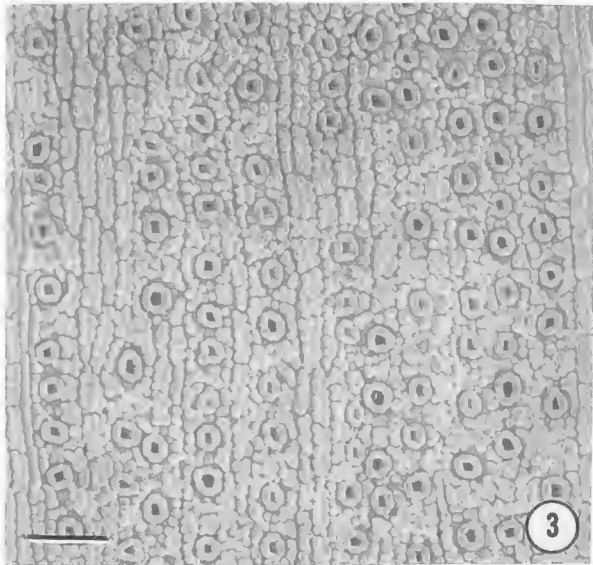
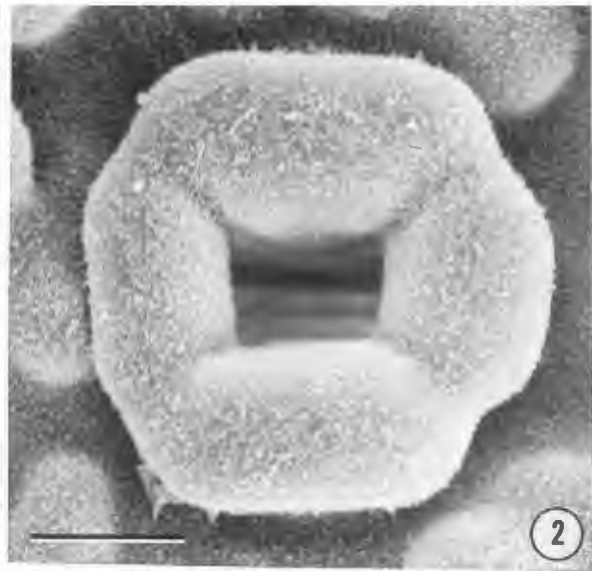
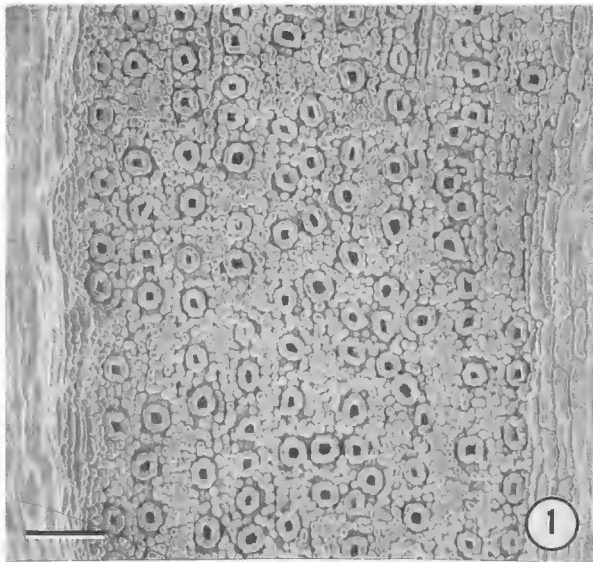
Since taxol was demonstrated to be a potent anti-cancer drug which was first isolated from the inner bark of Pacific yew, (Rowinski *et al.*, 1990; Wani *et al.*, 1971; Jijie *et al.*, 1991) attention has been focused on all aspects of the chemistry, biochemistry, and pharmacology of taxol and its related compounds, the taxanes. Initially, the most important source of taxol was the bark of mature Pacific yew, *T. brevifolia* (Chen *et al.*, 1994; Harvey *et al.*, 1991; Joel, 1994; Strobel *et al.*, 1993). However, more recent studies suggest that needles may have a higher taxol content than the bark (Choi *et al.*, 1995; Kwak *et al.*, 1995). Examples of other studies showing the presence of taxol and taxanes in other yew species are *T. baccata* (Appendino *et al.*, 1992a,b; Das *et al.*, 1993a,b; Elias and Korzhenevsky, 1992; Heaton *et al.*, 1993; Parmar *et al.*, 1993), the needles of which appear to be a good alternative source of taxol (Diergarten and Dreps, 1993; Gueritte-Voegelein *et al.*, 1994), *T. chinensis* (Zhang *et al.*, 1990; Zongping and Zhongjian, 1991), *T. cuspidata* (Fett Neto and Di-Cosmo, 1992), the hybrid made by man, *T. media* (Castor and Tyler, 1993; Chmurny *et al.*, 1993), and *T. wallichiana* (Barboni *et al.*, 1993; Georg *et al.*, 1993). The biosynthesis of taxol by the various yew species may also hold some interesting and important clues in *Taxus* taxonomy. For instance, Strobel *et al.* (1993) reported that C-14 acetate served as a precursor, in

Figures 1-6 (on the facing page). Scanning electron micrographs of *Taxus cuspidata* abaxial leaf surfaces. Figures 1-4. From an ornamental plant (in Provo, Utah): treated with Teepol detergent (Figs. 1 and 2); not treated with Teepol detergent (Figs. 3 and 4). Figures 5 and 6. Most recent year growth (from Pacific coast Oregon). Bars = 100 μ m (Figs. 1, 3 and 5) and 10 μ m (Figs. 2, 4 and 6).

vitro, for taxol in *T. brevifolia*, *T. floridana*, and *T. canadensis*, but in no other yew species. This work suggests that inherent biochemical differences do exist among yew species despite distinguishing morphological characters. As a result of the ever widening medicinal uses being found for taxol many laboratories have turned attention to the history, ethnopharmacology, botany, chemical composition, origin, chemical structure, properties, mechanisms of action, pharmacokinetics and other properties of *Taxus* (Mandrile *et al.*, 1993). Examples of relatively recent reviews include emphasis upon historical (Donehower and Rowinsky, 1993; Heinsteins and Chang, 1994; Suffness, 1993; Wall and Wani, 1994), clinical (Long, 1994; Pazdur *et al.*, 1993; Rowinsky *et al.*, 1993; Vyas, 1993), cell culture (Furusaki and Nakajima, 1994; Heinsteins and Chang, 1994), effects upon tubulin activity and cytotoxicity (Chazard *et al.*, 1994; Gueritte-Voegelein *et al.*, 1992; Kohler and Goldspiel, 1994; Long, 1994; Rothenberg, 1993), phytochemistry (Appendino, 1995; Das and Kashinath, 1996; Suffness, 1995), and procedures for determination and supply of taxol (Heinsteins and Chang, 1994). Although taxol has been synthesized (Nicolau *et al.*, 1994), the process is extensive and expensive. Perhaps a more promising approach may be to find ways to obtain taxol from certain yew associated fungi by fermentation methodology (Stierle *et al.*, 1994; Strobel *et al.*, 1996; Li *et al.*, 1996).

For some plant species epicuticular wax has a potential taxonomic significance (Ditsch *et al.*, 1995; Maffei, 1994; Chandrashekar and Sandhyarani, 1994; Theisen and Barthlott, 1994; Weiller *et al.*, 1994; Zygadlo *et al.*, 1994a,b). Plant leaf waxes have been studied for a variety of other reasons including acid rain (Kim and Soh, 1995; Percy and Baker, 1990; Nouchi, 1992), herbicide studies (Knoche, 1994), insect resistance (Eigenbrode *et al.*, 1991; Eigenbrode and Espelie, 1995; Stoner, 1990; Sugayama and Salatino, 1995), as a barrier against fungal attack (Schwab *et al.*, 1995), effects of UV-B radiation (Barnes *et al.*, 1996), water stress (Bondada *et al.*, 1996), and chemical composition as related to saline and non-saline habitats (Chandrashekar and Sandhyarani, 1994). McWhorter (1993) reported that wax weights on Johnson grass leaves varied

SEM of yew leaves



significantly with drought stress as compared to non-drought stressed plants, and other investigators have reported that cultivar and plant age can significantly affect quantity of wax (Guelz and Boor, 1992; Zaid, 1990). Young rolled leaves may have different composition from mature leaves (Guelz *et al.*, 1991), and Shiraishi (1990) observed waxes in the form of sand or rodlets during early stages of development with satsuma mandarin and the wax crystallized as amorphous platelets during later stages. Another important point is that tolerant species of cole crops can be protected from ammonium nitrate injury because of the presence of resistant epicuticular waxes (Bitterlich and Upadhyaya, 1990).

A standard procedure to remove epicuticular waxes is with hexane or chloroform or both (Bewick *et al.*, 1993). These procedures have been used for many plants (Boyer, 1996; Guelz, 1994; Johnson and Asay, 1995; Postbeittenmiller, 1996), and although free fatty acids, hydrocarbons, wax esters, aldehydes, fatty alcohols and fridean-3 α -ol, and alcohols have been found (Ahmad *et al.*, 1992), the prominent waxes are alkanes which differ from species to species. Stevens *et al.* (1994a,b) reported that triterpenes were prominent in waxes of *Sedum* species. Wax composition in young leaves just emerging from buds may differ from more mature leaves (Guelz *et al.*, 1991) and the amount and composition may vary when young emerging leaves are compared with older leaves (Guelz and Boor, 1992). Beech leaves contained mostly n-alkanals, n-alkanes and 1-alkanols (Reynhardt and Riederer, 1994), and dominant alkanes for *Coincya* were C29, C31, C27 and C29 (Vioque, *et al.*, 1994). On other plants, the odd-numbered carbon alkanes also predominated (Guelz, 1994; Osborne *et al.*, 1993; Zygadlo *et al.*, 1994a,b). The epidermis may also be covered with uniform wax layers without crystalloids (Guelz *et al.*, 1992).

Epicuticular wax surfaces can be observed with SEM or high resolution SEM (Barthlott *et al.*, 1996). The preferred methods are the use of low-temperature (Blanke *et al.*, 1994) cryofixation-cryosubstitution (Kim and Mahlberg, 1995) or low-temperature field emission SEM of frozen hydrated tissues. Roy *et al.* (1994) used low-temperature field emission SEM to demonstrate that normal artifacts which occur with dehydration and drying can be avoided, and hydrated well-preserved platelets of epicuticular leaf waxes can be observed. Unfortunately not many laboratories have both low-temperature equipment and field emission SEM, and with these procedures the best results are obtained with fresh tissues. When tissues are studied from various parts of the world it is very difficult to obtain fresh tissues for SEM studies. However, tissues are easily fixed and shipped in fixation solutions. Most studies of plant surfaces have

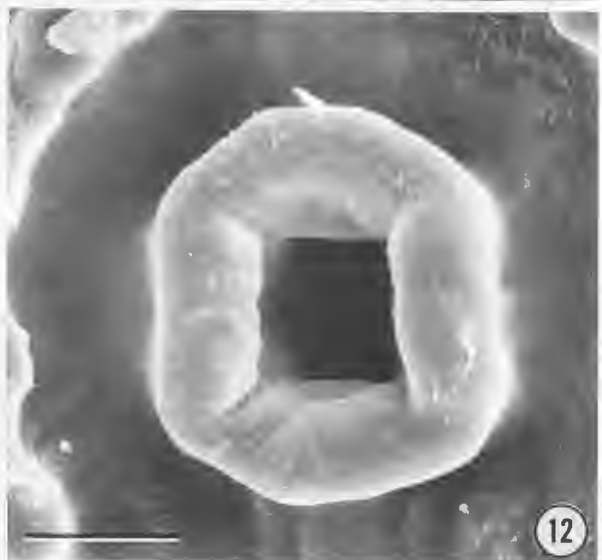
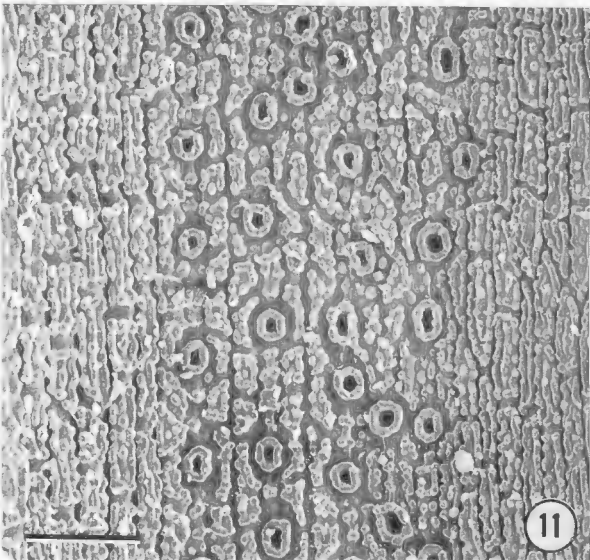
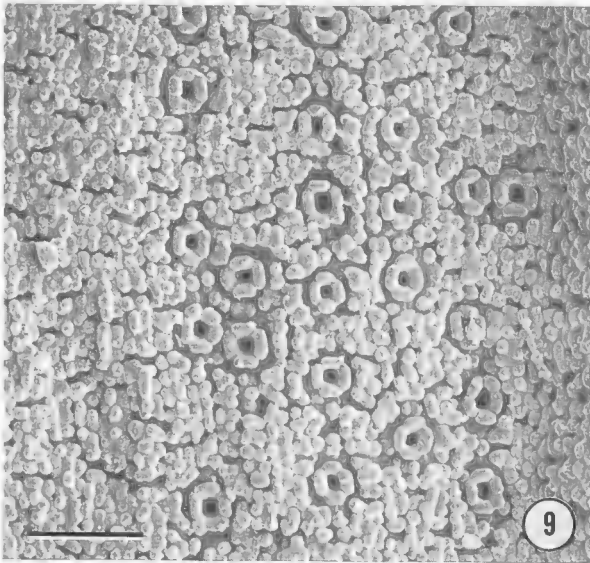
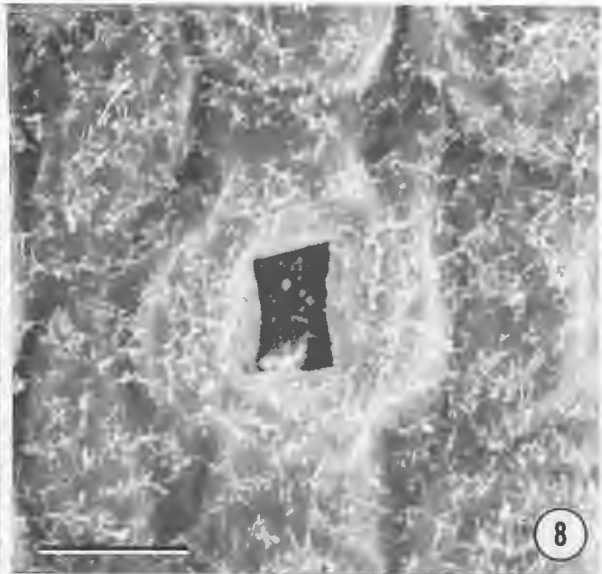
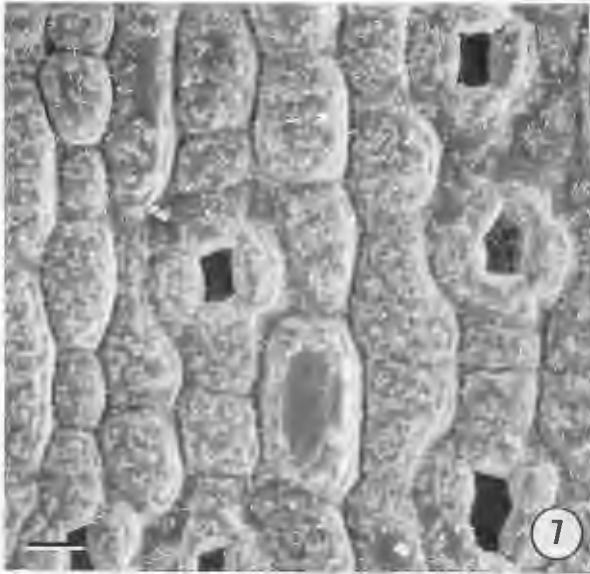
Figures 7-12 (on the facing page). Scanning electron micrographs of *Taxus* abaxial leaf surfaces. Figures 7 and 8. *T. cuspidata* from a young leaf showing subsidiary cell development (from Pacific coast Oregon). Figures 9-12. *T. brevifolia* from Northern Montana: from a 50 foot tree, approximately 1 foot stem diameter (Figs. 9 and 10); and from a small shrub in Glacier National Park, 2-3 cm diam, height 3-4 feet (Figs. 11 and 12). Bars = 10 μ m (Fig. 7, 8, 10 and 12) and 100 μ m (Figs. 9 and 11).

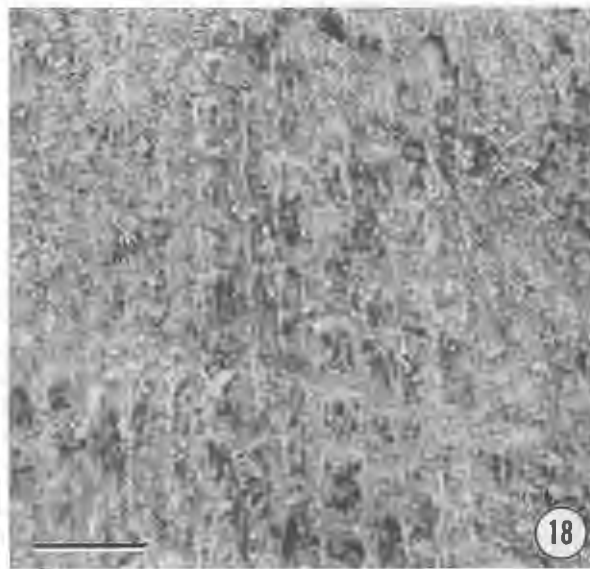
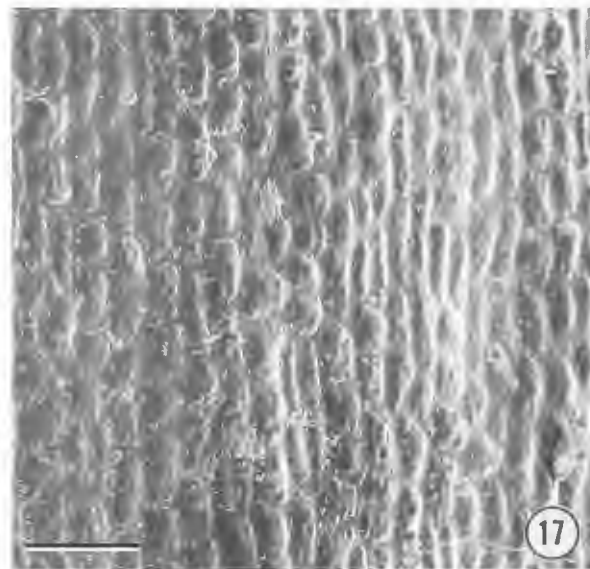
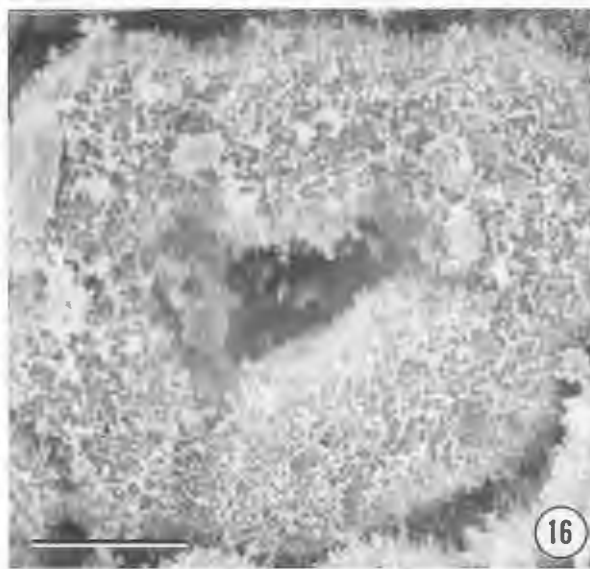
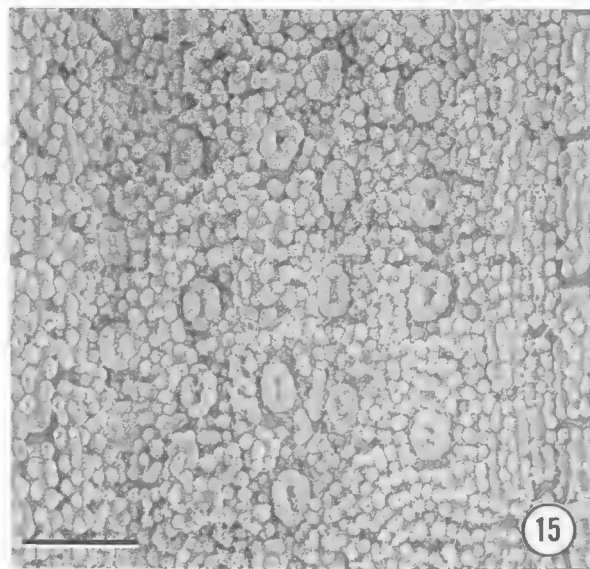
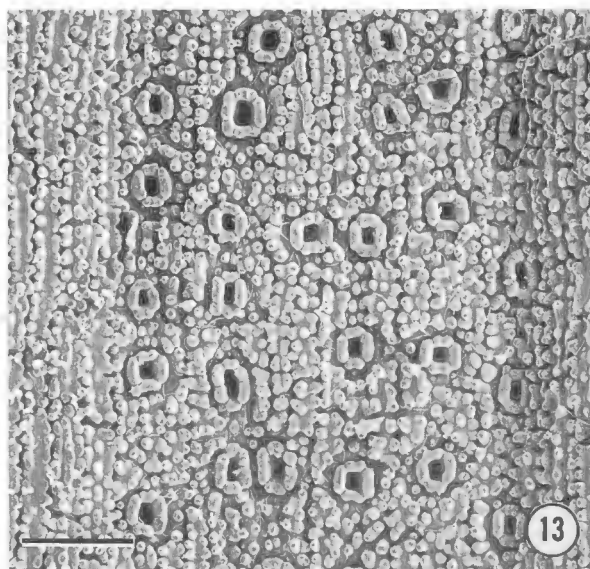
been completed with fixed tissues of critical point dried specimens without field emission SEM. The standard SEM procedures normally preserve the epicuticular wax structures on leaf surfaces. However, it must be recognized that critical point drying normally causes some tissue shrinkage which slightly distorts the surface morphology. This problem can normally be avoided with frozen hydrated tissues.

Since environmental and genetic differences are expected from plant to plant, significant variations in epicuticular wax patterns are to be expected even within a species. Using standard SEM procedures Kulshreshtha and Ahmed (1993) studied 16 species belonging to 13 genera of Euphorbiaceae and reported six main types of epicuticular wax: granular, crystalloid plates, ribbon, crust, rod-shaped, and aggregate. Variation in epicuticular crystalline structure can easily be visualized with SEM. Although the wax was sparse or inconspicuous for some species the predominant wax forms were fine granules and crystalloid plates. In other studies with *Sorghum bicolor*, Jenks (1993) reported 14 epicuticular wax classes. On leaf surfaces of some plants there is a continuous wax layer devoid of sculpturing or crystalloids (Guelz *et al.*, 1993; Mo and Wang, 1992; Vogel and Freeling, 1993) and Anton *et al.* (1994) reported diffusion or complex anastomosing pores as the mechanism of epicuticular wax deposition with broccoli. Additional studies with a variety of plants will help to elucidate questions about epicuticular waxes. Thus, it is of interest to use standard SEM procedures in order to determine whether different species of *Taxus* have different morphological types of waxes on leaf surfaces, to characterize stomatal and other leaf characteristics, and to relate these observations to *Taxus* taxonomy.

Although SEM photos have been published showing the leaf surfaces of a few yew species, no concerted attempt has been made to relate these observations to yew taxonomy (Strobel *et al.*, 1994). Most SEM studies on plants have focused upon pathogenic agents which invade plants through leaf surfaces. Since fungi constitute the largest group of plant pathogenic agents, there are more studies with fungi than with the other leaf surface

SEM of yew leaves





Figures 13-18 (on the facing page). Scanning electron micrographs of *Taxus brevifolia* leaf surfaces from Glacier National Park. **Figures 13 and 14.** Abaxial surface from a small plant in an ancient cedar forest. **Figures 15 and 16.** Abaxial surface from a shrub in an arid marginal area of the park at the southern limit of the range of growth. **Figures 17 and 18.** Adaxial leaf surfaces: from a 50 foot tree, approximately 16 inches diameter (**Fig. 17**) and from a small shrub in an arid marginal area of the park at the southern limit of the range of growth (**Fig. 18**). Bars = 100 μm (Figs. 13, 15, 17 and 18) and 10 μm (Figs. 14 and 16).

pathogens. Examples of recent investigations with fungi include cocoa fruits involving pathogen-host relationships (Flores *et al.*, 1994a,b), conidial germination, prepenetration structure formation, and postpenetration processes of *Alternaria porri* conidia on onion leaf surfaces (Aveling *et al.*, 1994), a report of mutant strains of conidia of *Fulvia fulva* with impaired ability to infect tomato cotyledons (Kenyon *et al.*, 1993), and studies of the infection process of *Coffea* by *Hemileia vastatrix* (Coutinho *et al.*, 1993). Examples of recent investigations with other leaf pathogens using electron microscopy include insects (McKenzie *et al.*, 1993), bacteria (Frankle, *et al.*, 1993; Van Doorn, *et al.*, 1994) and nematodes (Endo and Nickle, 1994).

Other recent investigations of leaf surfaces using scanning electron microscopy have included stomatal openings filled with dust (Kulshreshtha *et al.*, 1994a), studies with particles (Minarcic *et al.*, 1993), effects of auto exhaust upon leaf structure (Kulshreshtha *et al.*, 1994b), stomata on fruit surfaces (Burdon *et al.*, 1994; Cohen *et al.*, 1994; Potgieter and Van Wyk, 1994), wind damage on needle surfaces (Vangardingien *et al.*, 1991), and paleobotanical studies (Snigirevskaya, 1993), including *Taxus* (Hoeg, 1981).

Few recent studies have been reported concerning *Taxus* stomata although photosynthetic properties have been reported (Hager, 1967; Zajackowska *et al.*, 1982). Kartusch and Richter (1984) examined anatomical changes in *T. baccata* needles after an increase in the hydraulic resistance of the stem one year after a double sawcut was made in the stem. During the experiment, the number of stomata per unit area decreased, in contrast to previous literature reports. Given the status of current problems with yew taxonomy, and prospects of the limited history of leaf surface morphologies to aid in plant identification, we sought to use SEM as an aid to understanding yew taxonomy.

Materials and Methods

Leaf samples (growth from the previous year, unless

otherwise stated) were obtained from direct sampling of plants grown in field situations, or plant materials that were sent through express mail to our laboratories. Each specimen was immediately fixed and processed using methods of Upadhyay *et al.* (1991), except that tissues were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2-7.4). All tissues, except control samples, were treated with Teepol detergent in the fixative. Tissues were critical point dried, gold coated with a sputter coater and observed and photographed with either a JEOL 840A or a JEOL 6100 scanning electron microscope.

Results and Discussion

Abaxial leaf surfaces of *Taxus* plants have two guard cells which are characteristically overlaid by four subsidiary cells. Some plants also have distinctive epicuticular wax crystals. Since it was necessary to mail and transport fixed *Taxus* leaf tissues from different parts of the world, it was not possible to use low temperature scanning electron microscopy (SEM), an ideal procedure for studies of leaf surfaces (Blanke *et al.*, 1994). However, excellent images of plant surfaces can be obtained with conventional SEM procedures, particularly if wetting agents can be used to accelerate fixation. A question of concern was the effects of Teepol detergent upon epicuticular wax deposits. Therefore, samples were processed with Teepol detergent (Figs. 1 and 2) and without it (Figs. 3 and 4). These preliminary studies demonstrated that the morphological characteristics of epicuticular waxes of *Taxus* do not appear to be altered by the use of this commonly used wetting agent.

An additional question was whether it was necessary to use mature *Taxus* leaves for leaf surface studies. When mature *T. cuspidata* leaf surfaces, after one year of growth, from the Pacific Coast in Oregon (Figs. 5 and 6) were compared with young leaves (Figs. 7 and 8) it was obvious that immature leaves did not have the characteristic subsidiary cells located above the stoma, although epicuticular wax was evident both on immature and mature abaxial leaf surfaces.

Since *T. brevifolia* is an important source of taxol, this species is of particular interest. Subsidiary cell and epicuticular wax characteristics appeared to be similar when large trees (Figs. 9 and 10) were compared with small shrubs (Figs. 11 and 12) both from the middle of established forests. However, when leaf surfaces from small yew trees from an ancient cedar forest (Figs. 13 and 14) were compared with shrubby plants in an arid southern limit of the growth range, plants from the more arid areas had more distinctive epicuticular wax on the leaf surfaces (Figs. 15 and 16).

Although adaxial leaf surfaces of the various species

Table 1. A dichotomous key to the genus *Taxus* based upon surface features of leaves as observed with SEM.

-
1. One-half of abaxial side of the leaf having 3-5 rows of stomata.
 - T. floridana*, *T. canadensis*, *T. brevifolia*, and *T. globosa*
 1. Abaxial side of the leaf having raised individual papilliform epidermal cells between stomata ordered more or less in rows
T. floridana, *T. brevifolia*, and *T. globosa*
 2. Abaxial side of the leaf having what appear to be fused papilliform epidermal cells between stomata that are not raised
T. canadensis
 2. One-half of abaxial side of the leaf having 7-10 rows of stomata all other species
 1. Abaxial side of the leaf having raised individual papilliform epidermal cells ordered between stomata more or less in rows
T. wallachiana, and *T. sumatrana*
 2. Abaxial side of the leaf having more fused papilliform cells arranged in block-like patterns between the stomata
T. baccata, *T. cuspidata*, and *T. chinensis*
-

of yew were also examined with SEM, consistent differences were not evident except when adaxial leaf surfaces were examined comparing plants from large trees in the middle of the forest (Fig. 17) with small shrubs in the more arid southern limit of the growth range (Fig. 18). It was obvious that more dense epicuticular wax crystals were present on adaxial surfaces of leaves from the more arid regions.

It was assumed that the appearance of large deposits of epicuticular waxes on *T. brevifolia* growing in a relatively arid portion of the southern extreme limit of its range may be attributed to the environmental influences on wax production. The reasons for other wax pattern variations on yew leaf surfaces are unknown. For instance, commonly, the epicuticular wax pattern may vary from one area of a leaf to another and from one subsidiary cell to another on the same leaf. Also, the epicuticular wax patterns do vary slightly and indescribably from one species to another such as the case with the leaf surfaces of *T. floridana* (Figs. 19 and 20), *T. canadensis* (Figs. 21 and 22), and *T. globosa* (Figs. 23 and 24). All three are North American species. However, there is just as much, if not more, variation between the two specimens of *T. baccata*, one from Sweden (Figs. 25 and 26) and one from Norway (Figs. 27 and 28).

Even though the epicuticular wax differed morphologically between leaves from *T. wallachiana* (Figs. 29 and 30) and *T. sumatrana* (Figs. 31 and 32), general leaf characteristics were similar. It is obvious that epicuticular wax patterns can vary significantly even within the same species. As an additional example, the epicuticular wax patterns on leaves from *T. chinensis* differed significantly when young plants (Figs. 33 and 34) were compared with old plants (Figs. 35 and 36).

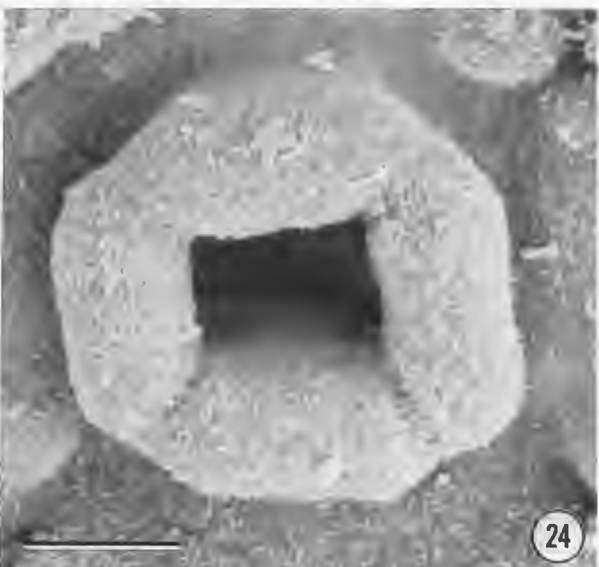
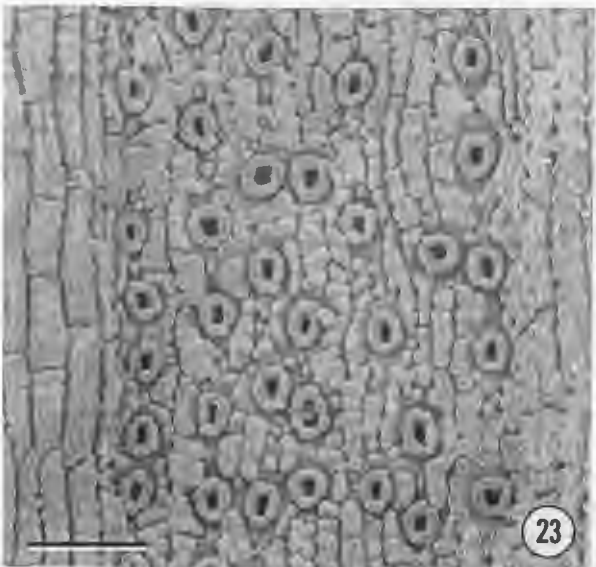
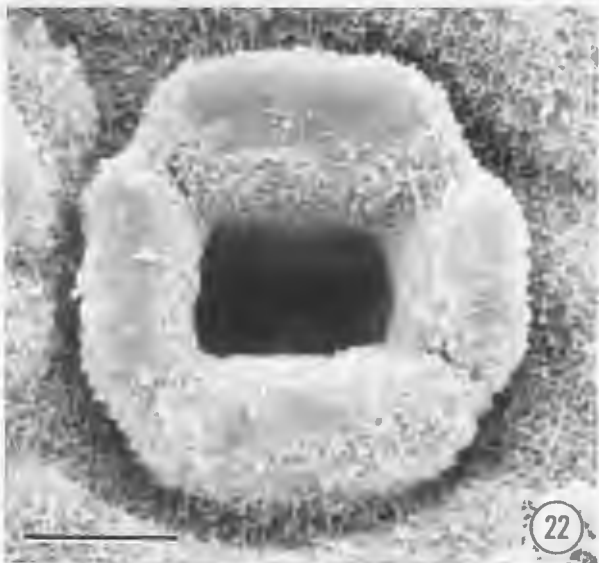
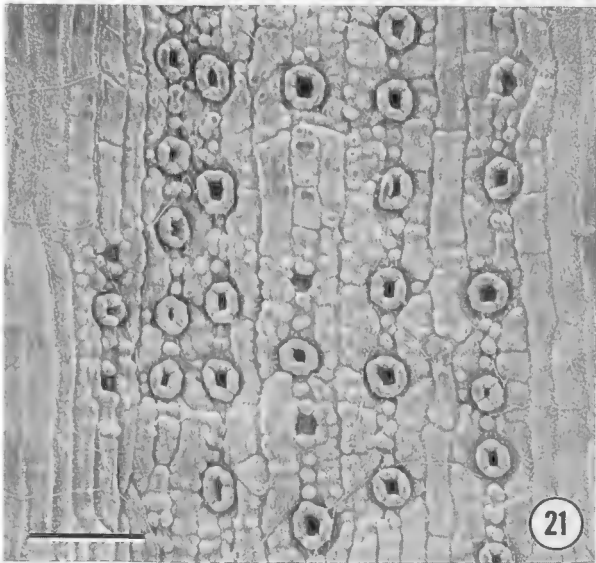
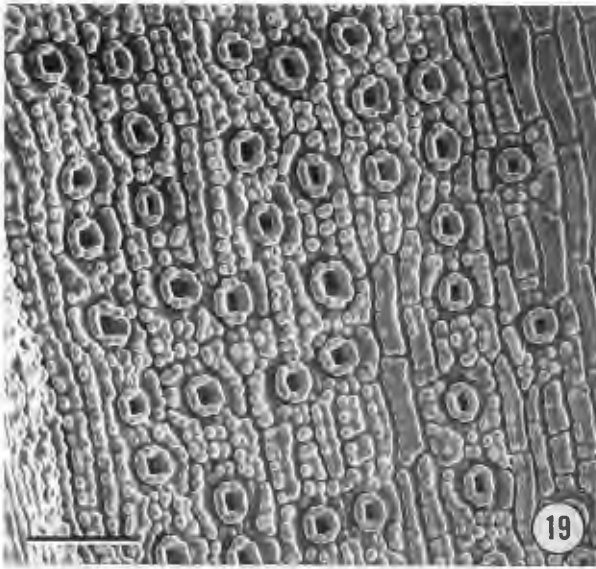
We observed what appeared to be environmentally, age, and location related differences in the epicuticular wax patterns on the surfaces of *Taxus* leaves. However, we could not find significant and consistent patterned

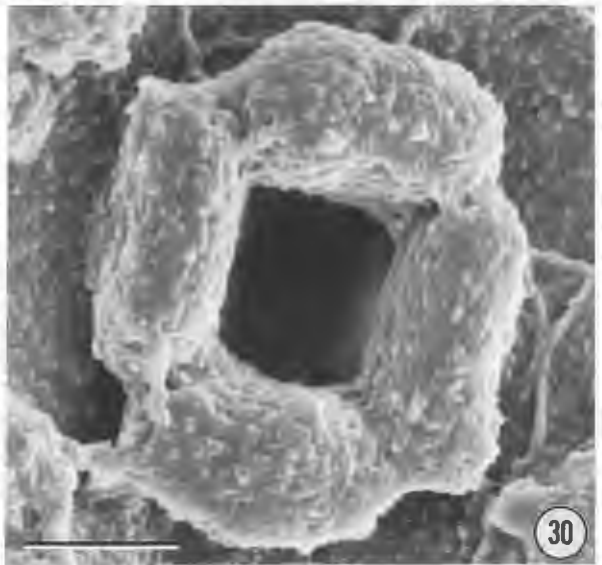
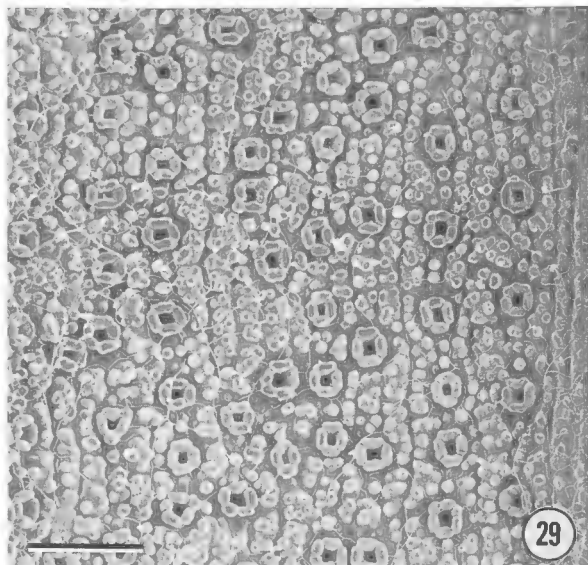
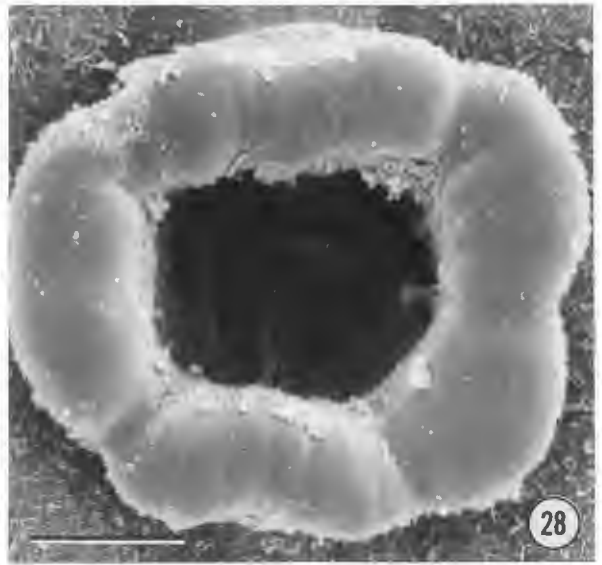
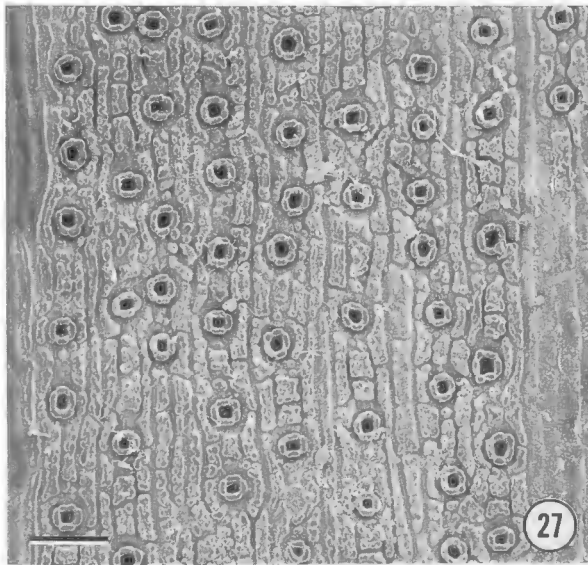
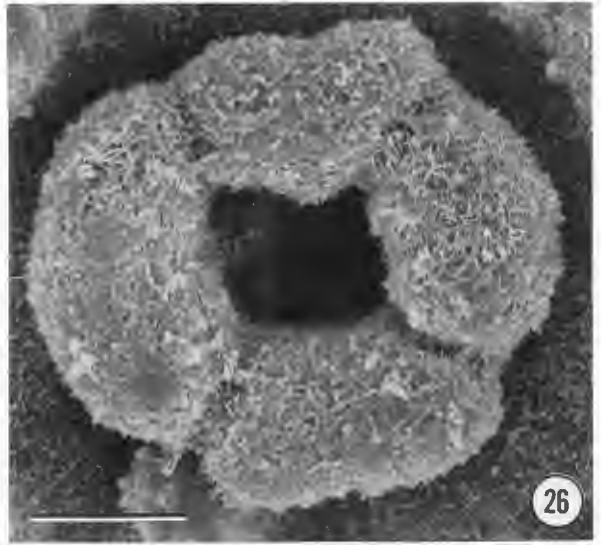
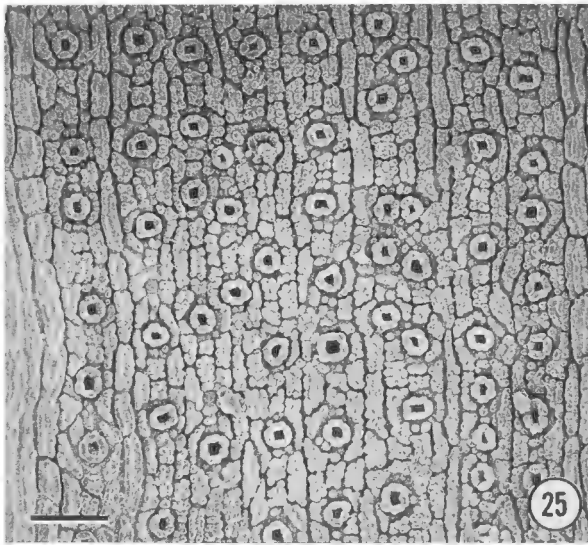
Figures 19-24 (on the facing page). Scanning electron micrographs of *Taxus* abaxial leaf surfaces. **Figures 19 and 20.** *T. floridana* from Northern Florida. **Figures 21 and 22.** *T. canadensis* from Canada. **Figures 23 and 24.** *T. globosa* from Mexico. Bars = 100 μ m (Figs. 19, 21 and 23) and 10 μ m (Figs. 20, 22 and 24).

differences between the designated species of yew. On the other hand, a very obvious difference between some of the yew species was observed which was the appearance of protruding papilliform epidermal cells on some species. This was especially evident on the Western yews, *T. brevifolia* (Figs. 9-18), *T. globosa* (Figs. 23 and 24) and *T. floridana* (Figs. 19 and 20), each of which has distinct protruding papilliform epidermal cells of the stomates. On the other hand, *T. canadensis*, another Western species, did not follow this pattern (Figs. 21 and 22). Its epidermal cells were only slightly protruding and appeared to be fused. Interestingly, the Himalayan yew, *T. wallachiana* (Figs. 29 and 30), and to a lesser extent, *T. sumatrana* (Figs. 31 and 32), have an appearance similar to the Western yews described above. The remainder of the yew species studied, including *T. cuspidata* from Japan and the Pacific coast of North America (Figs. 1-8), *T. baccata* from Europe (Figs. 25-28), *T. sumatrana* from Asia (Figs. 31 and 32), and *T. chinensis* from Asia (Figs. 33-36), have much less distinctive and more fused papilliform epidermal cells, best illustrated by *T. chinensis* (Figs. 33-36) and *T. baccata* (Figs. 25-28). The development of patterns of these papilliform epidermal cells seemed to be independent of age and environmental conditions of the plants.

Undoubtedly, the most consistent and significant observation of yew leaf surface morphology, as it relates to current proposed *Taxus* taxonomy, is that each of the native yew species from North America, including *T. brevifolia* (Figs. 9-18), *T. canadensis* (Figs. 21 and 22),

SEM of yew leaves





Figures 25-30. Scanning electron micrographs of *Taxus* abaxial leaf surfaces. **Figures 25-28.** *T. baccata* from Sweden (Figs. 25 and 26) and from Norway (Figs. 27 and 28). **Figures 29 and 30.** *T. wallichiana* from Nepal. Bars = 100 μm (Figs. 25, 27 and 29) and 10 μm (Figs. 26, 28 and 30).

T. globosa (Figs. 23 and 24) and *T. floridiana* (Figs. 19 and 20), have 3-5 rows of stomata on one-half of the abaxial leaf surface. Each of the other designated yew species found in other parts of the world consistently have 7-10 rows of stomata on one-half of the abaxial leaf surface (Spjut, 1992). Thus, although leaf surface wax patterns on *Taxus* show variation with regard to age, environmental, and perhaps species differences; the influences of the former factors seem to mask any consistent patterns that might be related to the latter influences. However, it seems that SEM observations of other features on yew leaf surfaces may prove useful in providing hints of taxonomic origin of any given plant. These include the number of rows of stomata, and the pattern and orientation of the intra stomatal papilliform-epidermal cells. When these two features of leaf morphology are combined an instructive dichotomous key can be developed which relates these two important characters to some of the currently accepted *Taxus* taxonomy (Table 1).

Commonly, given the absence of a sophisticated SEM for making leaf surface photographs to assist in *Taxus* identification, a simple but powerful hand lens or binocular microscope will prove useful in making these leaf surface observations.

We suggest that this study may provide some guidance to those taxonomists interested in yew species designations in geographical areas in which yew species seemingly either overlap or have interbred such as with *T. wallichiana* in the sub-continent of Asia and *T. baccata* whose range may extend across Eastern Europe into Asia (Hartzell, 1991). In addition, the leaf surface characters mentioned in this report may eventually assist in the clarification of yew species designations that have been given to a number of Asiatic species whose authenticity has been questioned (Hartzell, 1991). For instance, it is obvious that the raised papilliform cells of *T. wallichiana* allow it to be distinguished from the other asiatic yew *T. chinensis* and the European yew *T. baccata* (Table 1). Although it is obvious that these descriptors cannot be used to separate all of the species studied, they can, nevertheless, be useful to some degree in separating some of the species.

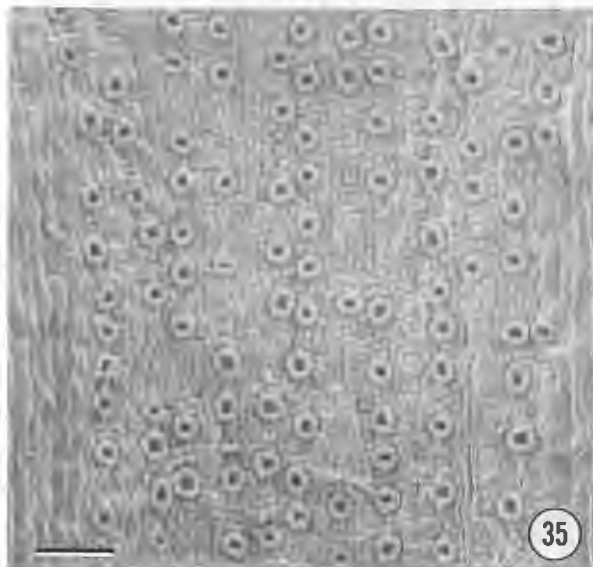
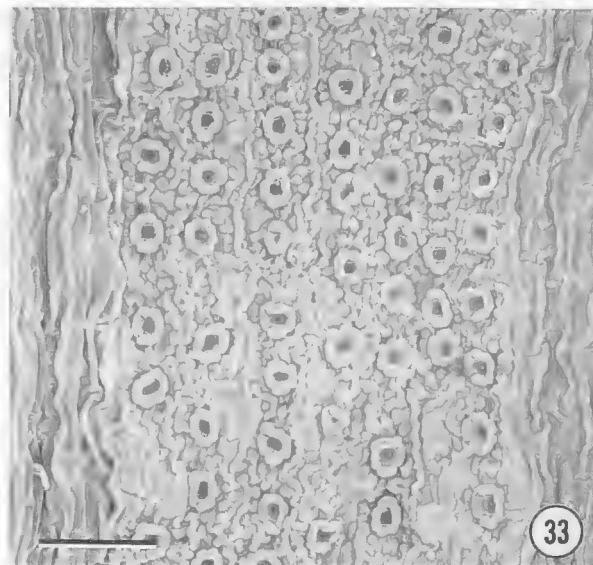
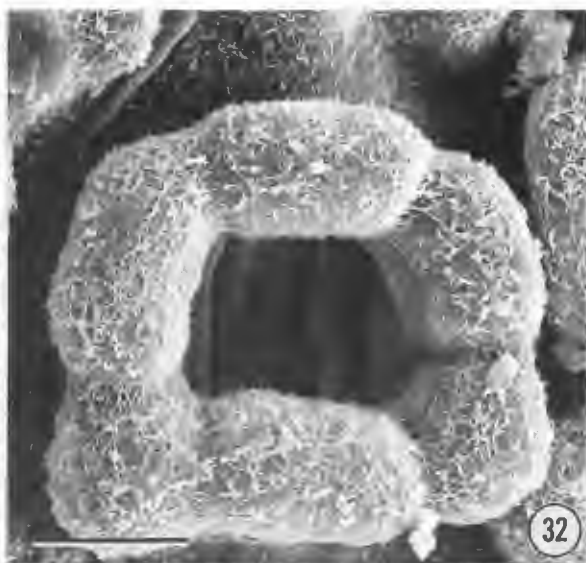
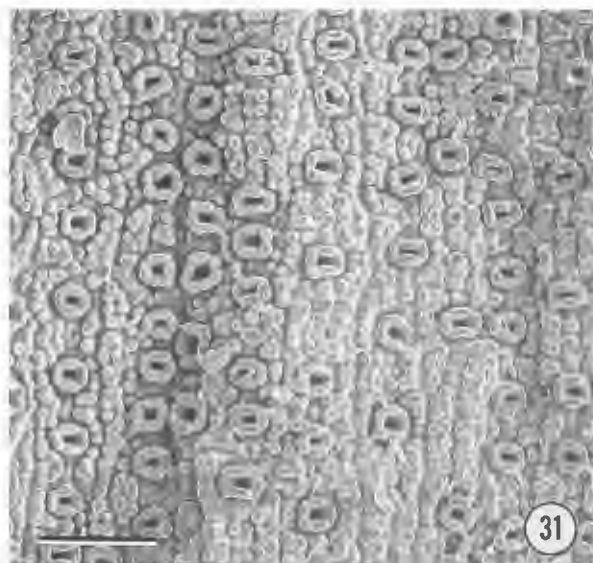
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Figures 31-36. Scanning electron micrographs of *Taxus* abaxial leaf surfaces. **Figures 31 and 32.** *T. sumatranensis* from the Philippines. **Figures 33-36.** *T. chinensis* from the Hangzhou Botanic Garden: from a young plant (**Figs. 33 and 34**) and from an 8 year old plant (**Figs. 35 and 36**). Bars = 100 μm (**Figs. 31, 33 and 35**) and 10 μm (**Figs. 32, 34 and 36**).

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.