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Nicolosi, Ralph Thomas

MORPHOLOGICAL FEATURES OF LEAVES AND POLLEN AS AN AID IN
SEPARATING SELECTED SPECIES AND CULTIVARS WITHIN THE GENUS
TAXUS

The Ohio State University

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Morphological Features of Leaves
and Pollen as an Aid in Separating Selected Species and Cultivars
within the Genus *Taxus*

Dissertation

Presented in Partial Fulfillment of the
Requirements for the Degree Doctor of Philosophy
in The Graduate School of The Ohio State University

By

Ralph Thomas Nicolosi, B.A., M.S.

***********

The Ohio State University

1982

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Co-Advisor
DEDICATION

This work is dedicated to my mother who would have been proud, and my father from whom I received an important education.
ACKNOWLEDGMENTS

This author expresses heartfelt gratitude to his advisor, Dr. R. Daniel Lineberger, for his patience, enthusiasm, and oft needed friendship and solace. In addition, to the members of the thesis committee, Dr. J. R. Geisman, Dr. F. O. Hartman, and Dr. R. A. Popham, for their scrutiny and guidance, I am indebted. For the everpresent willingness to help, and technical assistance of Mr. Robert Whitmoyer and his staff, I am grateful.

Finally, to John Kelly, with whom I have spent the last five years, for his ardent friendship, I am fortunate.
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Fields of Study

Major Field: Ornamental Horticulture
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Introduction

The Yew, whose mention dates back to the earliest Greek and Roman authors, has been the subject of exhaustive research as to its lineage, most recently by Chadwick and Keen (1976). Representing one of the most striking evergreen shrubs, the Yew is tolerant of a wide variety of soil conditions, and withstands pruning and shearing, making it serviceable as either hedges or specimens. In addition, because of the large number of named selections which vary in size, shape, and foliage coloration, the Yew can be used for numerous landscape needs, further reason its importance to the landscape industry is indisputable.

Ironically, however, because of its popularity, confusion surrounds many of the named varieties of *Taxus* as to their authenticity or distinctiveness. Due to their foreign origin, almost all of the earlier desirable forms were propagated and shipped from abroad. However, after the adoption of Quarantine 37 in 1918, importation of nursery stock ceased, while consumer demand for quality evergreens remained constant. As a result, American nurserymen propagated evergreens from any available source including seed from mixed plantings. In some cases, plants of unknown origin were named and marketed based solely upon what was in current demand.

There are currently in excess of 100 cultivated varieties of *Taxus*, the majority of which were selected for desirable growth habit and
hardiness; the remainder of which exhibit varying degrees of variegation. With few exceptions, immature containerized seedlings or cuttings are virtually indistinguishable simply because they fail to exhibit the distinctive growth habit for which they were originally selected. Consequently, the disorder that originally befell the genus and its varieties admittedly persists in the present day industry.

In 1942 the living herbarium of Taxus, as part of the Secrest Arboretum at the Ohio Agricultural Research and Development Center in Wooster was established in order to study the genus Taxus. As of 1976 the planting included 97 accessions including 5 species of which there were 92 cultivars. Since that time some have been removed due to severe winter injury or have died as a result of adverse cultural conditions. Much of this introductory information is based upon studies conducted by Chadwick and Keen, (Keen 1956; Chadwick and Keen, 1976) at the Secrest herbarium at Wooster, and this author is grateful to have had such a reliable and thorough groundwork on which to base his inquiry. There will be no attempt to further chronicle the ancestry of the Yew, as the work of the aforementioned authors remain definitively comprehensive. It is the purpose of this study, however, because traditional morphological characters alone have been unsatisfactory in clearly defining boundaries within a species, to examine morphological and ultrastructural characters of leaf and pollen surfaces as detected by scanning electron microscopy (SEM) for use as supplemental methods of cultivar distinction and separation within the genus Taxus. In addition, a descriptive study of the pollen wall characters will be conducted using transmission electron microscopy (TEM).
Method of Study

All plant samples used in this study were obtained from the Secrest Arboretum in accordance with the Finding List and Guide to the Secrest Arboretum (Spec. Circ. 91 rev.), Ohio Agricultural Research and Development Center (OARDC), Wooster, Dec. 1970. Replicate tip cuttings approximately 45-50 cm in length from the following accessions at their respective plot locations were collected on March 27, 1980 and April 7, 1981:

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<td>T. b. 'Erecta'</td>
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<td>T. b. 'Nigra'</td>
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</tr>
<tr>
<td>T. hunnewelliana 'Globosa'</td>
<td>A-31-104</td>
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\text{T. x media 'Amherst'} & A-31-53 \\
\text{T. m. 'Brownii'} & A-31-111 \\
\text{T. m. 'Burr'} & A-30-16 \\
\text{T. m. 'Hatfield 23'} & A-30-28 \\
\text{T. m. 'Ohio Globe'} & A-31-144 \\
\text{T. m. 'Wellesleyana'} & A-31-13 \\

The cuttings were placed in Floralife solution (9 g/l) in order to allow male strobili to open. Small quantities of the loose pollen were tapped free from open strobili onto polished aluminum disc stubs covered with doublefaced transparent tape. The stubs were stored dry in individual glass vials within a vacuum desiccator for later examination. In addition, pollen collected in 1978 and 1979 from some of the same accessions for a related study was used and will be discussed in Chapter 2.

Upon having collected pollen, the leaves were harvested and prepared for examination according to standard procedures as detailed in Materials and Methods.
CHAPTER 1
LEAF MORPHOLOGY

Literature Review

Plant classification schemes, whether they be natural, phylogenetic, or artificial, historically have been based upon gross morphological features. Both quantitative (presence or absence thereof), and qualitative (color and shape) descriptions of plant organs have served well to delimit genera and species. Intense cultivation, however, has resulted in many plant selections which, while differing genetically as to eventual growth habit or hardiness, exhibit only subtle phenotypic differences.

Albeit, strict and careful documentation of new plant introductions should preclude much misapplied nomenclature, yet, in any human endeavor, source for error is limitless. As new cultivated varieties are introduced, and as error mounts upon error, methods to supplement conventional classification must be investigated.

The increasing availability and sophistication in scanning electron and transmission electron microscopy continue to make electron optics invaluable to horticultural application. Enabling the scientist to resolve more detail and see what has otherwise remained beyond the limits of light microscopy, advances in a wide variety of plant disciplines have been made possible (Aist and Williams, 1971; Baker and Parsons, 1971; Falk, et al., 1970; Hess, et al., 1974; Laetsh, 1974;
Vesk, et al., 1966). Scanning electron microscopy (SEM) is a versatile technique by which many surfaces of plant parts can be studied (Baker and Parsons, 1971; Holloway, 1971; Rasmussen and Hooper, 1974). Sections of up to 0.5 cm can be examined with ease over a magnification range from 20x to 20,000x, requiring uncomplicated methods of specimen preparation (Carr, 1971; Holloway, 1971; Rasmussen and Hooper, 1974). In addition, Stockey and Taylor (1978) in their comparison of epidermal patterns in the genus Araucaria, noted that there was no distortion of fine feature during specimen preparation as did Baker and Parsons (1971) in their assessment of artifacts such as shrinkage or deformation.

The leaf has been the subject of investigative study using light and scanning electron microscopy in documenting information on both the physical (Bahadur, 1971; Cutler and Brandham, 1976; Kausik, 1974; Miranda, 1970; Pant and Verma, 1974; Raju and Rao, 1977; Shah, et al., 1972; Sharma and Butler, 1975), and the chemical (Buckovac and Widmoyer, 1980; Hallam, 1971; Holloway, 1971; Hanover and Reicosky, 1971; Hartmann, 1978), characteristics of its surface. Furthermore, much of these data have been successfully applied to systematic classification at the familial (Ahmad, 1974; Alvin, 1974; Bahadur, et al., 1971; Baily, et al., 1980; Shah, et al., 1972), generic (Buckovac and Widmoyer, 1980; Hanover and Reicosky, 1971; Miranda, 1980; Stockey and Taylor, 1978), specific (Korn, 1972; Pant and Verma, 1974; Sharma, et al., 1981; Stace, 1969), and intraspecific (Brandham and Cutler, 1978; Coon, 1971; Krause, 1976; Martens and Fretz, 1980) levels.
Cutler and Brandham (1976) in their work with hybrid Aloe suggested strong genetic control over leaf pattern, including stomate position and presence or absence of papilla. In a study of stomate position and epidermal sculpturing, F₁ hybrids were intermediate in both traits to their parents and, moreover, uniform among themselves. The same two authors later published that hybrids between Aloe rauhii and A. dawei with either numerical or structural chromosomal aberrations could be distinguished from epidermal characters based on stomate size, and subsidiary cell size and shape (1978). Furthermore, epidermal characters such as stomata appear to be orderly arranged, and follow a species-specific process for induction of stoma initials (Korn, 1972; Marx and Sachs, 1977).

The systematic application of microtopographical features is well documented. In studying the organizational variations of the stomatal complex in monocotyledons, Stebbins and Khush (1961) were able to correlate differences to type of seed germination, vascular anatomy, growth habit of mature plants, and geographic locations. Likewise, variation in the structure and development of foliar stomata in 50 species of 28 genera in the family Euphorbiaceae proved to be taxonomically significant (Raju and Rad, 1977). Ahmad (1974) demonstrated that stomatal size and density could confirm subfamily organization in the family Acanthaceae. Within the genus Araucaria two distinct groups could be recognized based upon stomatal orientation and number of subsidiary cells (Stockley and Taylor, 1978). At the intraspecific level, stomatal frequency and size aided in the
distinction between cultivars of crabapple (Martens and Fretz, 1980) and roses (Coon, 1971).

In addition to stomata themselves, the width of stomatiferous and non-stomatiferous areas and quantitative characters such as number of stomatal rows per band enabled Pant and Verma (1974) to determine species within the genus Ephedra. Additional features such as hairs (Ahmad, 1974; Krause, 1976; Martens and Fretz, 1980; Stace, 1969), epidermal topography (Coon, 1971; Cutler, 1979; Hartmann, 1978; Pant and Verma, 1974; Stockey and Taylor, 1978), and cuticular properties (Miranda, 1980; Pant and Verma, 1974; Sharma, et al., 1981), have been cited for use in and of themselves or in conjunction with other characters for use as taxonomical tools.

Although environmental effects have been shown to alter stomatal frequency in clover, stomata size and the arrangement of the subsidiary cell complex remained stable and reliably useful for classification (Sharma and Butler, 1975). And, while other authors caution that some intrageneric and intraspecific variation may exist in features such as wax density, it is generally agreed upon that structural and ontogenetic stability persists (Brandham and Cutler, 1978; Cutler and Brandham, 1976; Hartmann, 1978; Krause, 1976; Shaw, et al., 1972; Stockey and Taylor, 1978).
Materials and Methods

The cultivars used in this investigation are set out in the Introduction to this study together with their herbarium accession number.

Scanning Electron Microscopy (SEM) Specimen Preparation

One-year-old mature leaves from each sample were excised from stem tips approximately 6.0 to 12.0 cm from the apex. Midsections approximately 0.4-0.5 cm in length of 3 leaves each from replicate samples were enclosed in gauze bags and labeled according to their identity. The samples were fixed in 3.5% gluteraldehyde in 0.1M Tricine buffer at pH 7.2 during vacuum infiltration for approximately 15 minutes. Specimens were then dehydrated in ethanol concentrations for 30 minutes in each concentration with one change of liquid in each of the following: 25, 50, 75, 95, 100, 100% ethanol. Each was then transferred to (1:1 v/v) amyl acetate-ethanol mixture for 60 minutes and finally held in 100% amyl acetate until critical-point dried with liquid CO₂.

Replicate samples were also snap frozen in liquid nitrogen, rapidly transferred to a pre-frozen desiccator and stored at -30 °C.

Three sections of each sample were attached to SEM stubs with conductive cement and sputter-coated with approximately 200 A platinum in a Polaron Equipment Limited SEM Coating Unit E5100. The samples were
examined on an International Scientific Instrument ISI-40 SEM at 30 kv. Samples that were fixed in gluteraldehyde and liquid nitrogen were also examined in order to compare fixation procedures.

Coating and SEM examination took place in the Electron Microscopy Laboratory, Department of Plant Pathology, OARDC, Wooster, Ohio during the time between April and September 1981.

Light Microscopy (LM) Specimen Preparation

Plastic epidermal imprints as described by Williams (1973) were prepared for each cultivar. Five one-year-old mature leaves from each cultivar, previously snap frozen in liquid nitrogen and stored at -30 °C, were painted with a clear lacquer on the abaxial surface. After a sufficient drying time of approximately 30 minutes the plastic peels were carefully removed using forceps and free mounted on glass microscope slides. Cover slips were loosely attached at the margins so as to reduce distortion of the plastic imprints.

The samples were examined and photographed using a Zeiss Photomic III photomicroscope under the scanning objective and working micrographs were printed at a final magnification of 40x. Micrographs of 5 leaves from each sample were examined; the parameters of interest being stomate density expressed as number per cm², and stomatal band width expressed in microns (um).

A clear acetate overlay outlined with 6 separate fields of reference at arbitrary points (3 on each band) was used to facilitate counting. Likewise, width measurements were taken at 6 distinct points
on each micrograph. Data expressed below represent mean values from 6 measurements or counts of 5 micrographs of each cultivar. Where differences occur, means are declared significantly different by use of Duncan's Multiple Range Test at the 5% level.
RESULTS AND DISCUSSION

Gross Morphology

The *Taxus* leaf, linear and often falcate in shape ranges in length from 16-25 mm and 2.0-2.5 mm in width (Fig. 1A). They are attached to the branches by short petioles spirally, but often pectinate, appearing 2-ranked. The adaxial surface is dark lustrous green while the underneath surface appears yellowish green. Two broad stomatal bands are evident on the underneath side of the leaf while the only prominent feature on the adaxial surface is a centrally located unbranched midvein (Fig. 1B). The margins are entire, and it is the only single veined conifer leaf without resin ducts (Esau, 1977). The leaves vary in thickness and are generally fleshy.

**SEM Observation: Adaxial Leaf Surface Characters**

The outer (periclinal) walls of the epidermal cells appear slightly convex, devoid of any conspicuous pattern (Fig. 2A). Papillae are absent; however, pronounced intercellular ridges for a moderately reticulate relief. The outermost peripheral wall configurations are regular, more or less rectangular in shape, approximately 50 um by 15 um in dimension (Fig. 2A). No stomata were observed on the adaxial surface of any of the leaves examined, suggesting that the 5 species involved in this study are hypostomatic. Wax crystals are fine and irregular (Fig. 2A).
The periclinal walls along the leaf margin are moderately convex, forming lumps which grade inconspicuously into the domed wall from which they arise (Fig. 2B). Throughout the five species, they occur in either one or two rows.
Figure 1. Gross morphological features of *Taxus media* 'Brownii'

Unbranched midvein marked by arrow.
Figure 2. Adaxial and marginal features of a *Taxus* leaf that had been critical-point dried.  A) *Taxus cuspidata* 'Intermedia' adaxial surface. Magnification bar equals 40 um.  B) *Taxus hunnewelliana* leaf margin. Magnification bar equals 300 um.
Abaxial Leaf Surface Characters

The abaxial surface is well defined into stomatiferous and non-stomatiferous regions (Fig. 3A). Two broad stomatiferous bands demarked by strongly convex periclinal epidermal walls occupy nearly 75% of the surface. The non-stomatiferous region, including the midvein area, is similar to the adaxial surface, exhibiting slightly convex outer epidermal walls interposed with continuous ridges (Fig. 3B). Conversely, the stomatiferous region is marked by conspicuous convolute epidermal walls forming a reticulum (Fig. 3A). The surfaces arise abruptly with an apparent constriction between the base of the protrusion and the cell wall (Fig. 4A). The cell surfaces are smooth, sometimes arranged in rows appearing to fuse intermittently (Fig. 4C), or randomly situated between and amongst the stomatal complexes (Fig. 4B).

It should be noted here that other authors (Cutler, 1979; Cutler and Brandham, 1976; Hartmann, 1978) regard any noticeable projection from the cell wall that is equivalent to at least 1/6 of the width of the cell in diameter, as papillae. That term, however, does not seem appropriate to this particular descriptive study.

In order to assess whether or not the lump-like projections were due in part, or altogether, to epicuticular wax deposits, samples were agitated in organic solvents capable of dissolving long-chain
Figure 3. Dual magnification micrographs of the abaxial surface of *Taxus* leaves that had been critical point dried.

A) *Taxus hunnewelliana*. Magnification bar equals 1200 μm in the left portion and 300 μm in the right. B) *Taxus baccata 'Dovastoniana.' Magnification bar equals 150 μm in the left portion and 50 μm in the right.
Figure 4. Leaves from *Taxus media* 'Amherst' that were agitated in various organic solvents. A) Snap frozen in liquid nitrogen. B) Xylene. C) Acetone. D) Chloroform. Magnification bar equals 2 um.
aliphatic compounds of which surface waxes are comprised (Holloway, 1971). As can be seen in Figure 4, there are no discernible morphological differences between an untreated leaf surface (Fig. 4A) and those subjected to three organic solvents: xylene, acetone, and chloroform (Fig. 4B, C, D, respectively). Furthermore, it can be clearly seen in Figure 5 that the structures previously referred to arise from a well-defined epidermal layer.

Stomata are sunken and have above them a suprastomatal cavity (Fig. 6A) with each stomata consisting of two sunken guard cells (g) (Fig. 6B). The epidermal cells adjacent to the guard cell pair are regarded as subsidiary cells because they differ in their morphology and orientation from other epidermal cells not associated with stomata (Fig. 6). Distinct in their strongly convex surfaces, they range in number from 4 to 6. It is the entire stomatal complex delineated by its subsidiary cell arrangement to which a later quantitative analysis will refer.

Morphological features as previously described refer generally to all of the species and their cultivars. Anatomical observations, such as stomate orientation and size, subsidiary cell number or configuration, variation in cell surfaces, failed to demonstrate that these features are under strong genetic control within the genus Taxus, as no observable structural differences could be detected among the five species studied. Furthermore, in a comparison of a cultivar of T. hunnewelliana which is a hybrid between T. canadensis and T. cuspidata, to cultivars of each of its parents, no intermediacy in leaf
Figure 5. A leaf from *Taxus baccata* 'Nigra' in which the epidermal layer is exposed in cross section. Magnification bar equals 100 um. Arrow designates epidermal layer of the adaxial surface.
Figure 6. Stomatal complexes including subsidiary cells, suprastomatal cavity, and guard cells of *T. media* 'Brownii.' A) Entire complex. B) Complex partially fractured revealing sunken guard cells. Magnification bar equals 10 μm. Subsidiary cell (S), guard cell (G), epidermal layer, arrow.
surface morphology could be detected as reported by Cutler and Brandham (1976) in their study of Aloe hybrids. Similarly, in a comparison of $T. \text{media}$ cultivars to the species parents, $T. \text{cuspidata}$ and $T. \text{baccata}$, no perceivable morphological relationship between them is evident. It must be recognized that Cutler in his Aloe study was working with $F_1$ generations whereas in this study representative cultivars from their respective species were compared due to the fact that sources of the straight species are not available. It is, therefore, entirely possible that because of the subtle genotypic variation in cultivars within the species, differences between species that otherwise would be detectable, have been masked as a result of intense cultivation. On the other hand, the differences between the Yews are so small that early taxonomists considered them to be a single species (Keen, 1956) and only later separated them based upon geographical range. Consequently, their inherent genetic similarities may preclude any perceptible morphological variation in leaf surface patterns.

As noted previously in the scanning electron micrographs, the abaxial surface of the Taxus leaf is defined into stomatiferous and non-stomatiferous regions as delimited by variation in cellular shape. In addition, the individual stomata are recognizable by their subsidiary cell arrangement. Plastic epidermal imprints, as described in Materials and Methods, which provide a visible impression of these cellular variations were employed in order to assess whether or not leaf surface features were of classificatory significance. Without regard for cuticular structure, stomatal band width and stomate frequency as delineated by cellular structure were examined.
Light micrographs of plastic epidermal imprints are presented for all of the samples in Figures 7, 8, and 9. It is from micrographs such as these that quantitative data were calculated for stomate frequency per unit area, and stomatal band width.

Coon (1971), in her study of epidermal characters for the identification of *Rosa hybrida* cultivars, thoroughly reviewed various techniques and methods of obtaining epidermal imprints by using molds of various composition. It is acknowledged that regardless of the material, whether it be cellulose acetate, rubber, or any commercial preparation, a certain amount of shrinkage or distortion cannot be avoided. Recognizing this fact, sample comparison counts were conducted from SEM micrographs and these values differed no more than 5% from values obtained from the plastic epidermal prints (Table 1).

Based upon data obtained from epidermal plastic imprints as described earlier, all 5 species were declared significantly different from one another in a comparison of stomatal band widths, whereas 4 differed with reference to stomate density (Table 2). The difference between *Taxus hunnewelliana* which exhibits the widest band width (546 um) and *T. canadensis* with the narrowest band width (321 um) is readily apparent in Figures 7D and 8. The extremes, however, between the species with the highest and lowest stomate density, *T. cuspidata* (Figure 8) and *T. hunnewelliana* (Figure 7), respectively, are not as visually obvious.
Figure 7. Plastic epidermal imprints from the abaxial surface from which stomate and band width calculations were made.

A) \textit{Taxus baccata} 'Aurea.' B) \textit{Taxus baccata} 'Nigra.'

C) \textit{Taxus baccata} 'Dovastoniana.' D) \textit{Taxus hunnewelliana}.

E) \textit{Taxus baccata} 'Erecta.' F) \textit{Taxus canadensis}.

Magnification bar equal 0.5 mm.
Figure 8. Plastic epidermal imprints of cultivars within *Taxus cuspidata*. A) 'Adams,' B) 'Prostrata,' C) 'Hiti,' D) 'Intermedia,' E) 'Nana,' F) 'Thayerae.' Magnification bar equals 0.5 mm.
Figure 9. Plastic epidermal imprints of cultivars within *Taxus media*. A) 'Amherst,' B) 'Hatfield 23,' C) 'Brownii,' D) 'Ohio Globe,' E) 'Burr,' F) 'Wellesleyana.' Magnification bar equals 0.5 mm.
As with morphological features, there is no intermediacy in either stomate density or band width for hybrids. *T. hunnewelliana* possesses a markedly lower stomate density and larger band width than either of its parents *T. canadensis* and *T. cuspidata*, as does *T. media* in comparison to its parents *T. cuspidata* and *T. baccata* (Table 2).

As reasoned previously, this relationship reported by previous investigators (Brandham and Cutler, 1978; Cutler, 1979) whereby *Aloe* hybrids were intermediate to their parents in leaf surface characters, may be masked due to the fact that subsequent generations of cultivars are being compared rather than straight species. Equally as important to consider is the fact that the values for *T. canadensis* and *T. hunnewelliana* were compiled from one variety each, whereas values for *T. cuspidata* and *T. media* were compiled from six varieties each, and values for *T. baccata* were compiled from four varieties. Because of this, a general linear model, which allows for statistical comparisons of samples of unequal size, was employed in the statistical analysis. Therefore, although the data in Table 2 present a statistically valid comparison, the interpretation is open to dispute.

Furthermore, the data in Table 2 must be considered in light of comparisons of cultivars within individual species. For example, in Table 3 there is a clear distinction among all of the cultivars within *T. cuspidata* based upon both band width and stomate density. However, if each of the values are assessed individually, *T. cuspidata* 'Intermedia' with a stomate value of 14,183 falls into the range of the
Table 1. Comparative values for stomate density calculated from SEM micrographs and epidermal imprint light micrographs.

<table>
<thead>
<tr>
<th>Species</th>
<th>SEM</th>
<th>Light Micrograph</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. canadensis</td>
<td>13918</td>
<td>14442</td>
</tr>
<tr>
<td>T. hunnewelliana</td>
<td>12041</td>
<td>13183</td>
</tr>
<tr>
<td>T. media 'Amherst'</td>
<td>9373</td>
<td>9016</td>
</tr>
<tr>
<td>T. baccata 'Aurea'</td>
<td>18870</td>
<td>19524</td>
</tr>
</tbody>
</table>
Table 2. Stomatal band width and stomate density for five species of *Taxus*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Band width (um)</th>
<th>Stomate density (#/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. canadensis</em></td>
<td>321 a²</td>
<td>14442 a</td>
</tr>
<tr>
<td><em>T. hunnewelliana</em></td>
<td>546 b</td>
<td>13183 b</td>
</tr>
<tr>
<td><em>T. baccata</em></td>
<td>444 c</td>
<td>18439 c</td>
</tr>
<tr>
<td><em>T. media</em></td>
<td>513 d</td>
<td>14365 a</td>
</tr>
<tr>
<td><em>T. cuspidata</em></td>
<td>470 e</td>
<td>21479 d</td>
</tr>
</tbody>
</table>

²Mean separation by Duncan's multiple range test, 5% level.
species value for *T. canadensis* (14,365) rather than *T. cuspidata* (21,479). The same holds true based upon the band width values.

Subsequent examination of data in Table 3 in which cultivars within *T. baccata* and *T. media*, respectively, are compared, further illustrates that the interspecific variation that appears in Table 2 is precluded by the intraspecific variation. *T. baccata 'Erecta'* with a stomate value of 23,847 (Table 3) is closer numerically to *T. cuspidata* (Table 2), while *T. media 'Brownii'* at 12,946 (Table 3) is closest to *T. hunnewelliana* with a value of 13,183 (Table 2). Based upon these observations, there is obvious overlap of values from a cultivar of one species into another species.

In Table 3, in which cultivars of *T. baccata* are compared, 'Aurea' and 'Nigra' are not statistically different from one another based upon their band width and stomate density values. In fact, the values are strikingly similar to one another. It is of interest to note that *T. baccata 'Aurea'* has been widely grown from seed to avoid a lopsided plant due to plagiotropic growth and considerable latitude has been given to the requirements governing its growth habit as long as the underneath side of the leaves are yellow. On the other hand, *T. baccata 'Nigra,'* whose origin and growth habit are equally as vague, is characterized as having dark green leaves and having an upright spreading growth habit with ascending branches. It is entirely possible that these two plants are more similar than previously suspected with the exception of foliage coloration. Whereas the author recognizes
Table 3. Stomatal band width and stomate density for cultivars within 
*T. cuspidata*, *T. baccata*, and *T. media*.

<table>
<thead>
<tr>
<th>T. cuspidata</th>
<th>Band width (um)</th>
<th>Stomate density ($#/cm^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Prostrata'</td>
<td>588 a</td>
<td>22335 a</td>
</tr>
<tr>
<td>'Hitri'</td>
<td>536 b</td>
<td>24382 b</td>
</tr>
<tr>
<td>'Adams'</td>
<td>467 c</td>
<td>16171 c</td>
</tr>
<tr>
<td>'Nana'</td>
<td>435 d</td>
<td>31499 d</td>
</tr>
<tr>
<td>'Thayerae'</td>
<td>415 e</td>
<td>20299 e</td>
</tr>
<tr>
<td>'Intermedia'</td>
<td>381 f</td>
<td>14188 f</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T. baccata</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>'Aurea'</td>
<td>492 a</td>
<td>19524 a</td>
</tr>
<tr>
<td>'Nigra'</td>
<td>487 a</td>
<td>19418 a</td>
</tr>
<tr>
<td>'Dovastoniana'</td>
<td>425 b</td>
<td>10968 b</td>
</tr>
<tr>
<td>'Erecta'</td>
<td>372 c</td>
<td>23847 c</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T. media</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>'Wellesleyana'</td>
<td>605 a</td>
<td>18926 a</td>
</tr>
<tr>
<td>'Ohio Globe'</td>
<td>564 b</td>
<td>12725 b</td>
</tr>
<tr>
<td>'Amherst'</td>
<td>508 c</td>
<td>9016 c</td>
</tr>
<tr>
<td>'Burr'</td>
<td>506 c</td>
<td>15048 d</td>
</tr>
<tr>
<td>'Brownii'</td>
<td>458 d</td>
<td>12946 b</td>
</tr>
<tr>
<td>'Hatfield 23'</td>
<td>438 e</td>
<td>17531 e</td>
</tr>
</tbody>
</table>

*Mean separation by Duncan's multiple range test, 5% level.*
that, based upon the current data, it would be grossly premature to attempt to reorganize and reclassify the above mentioned cultivars, they remain the only two that are indistinguishable, based upon both stomate density and band width.

There does not seem to be a relationship between stomate density and band width in any of the comparisons. That is, smaller band widths do not always contain the highest density of stomates per unit area or vice versa. For example, T. hunnewelliana, whose cultivars averaged the widest band width, averaged the lowest stomate density (Table 2). Similarly, with isolated exceptions, there is no reliable correlation in the cultivar comparisons found in Table 3.
Summary

Leaves from the species and cultivars of *Taxus* that were examined in this study are morphologically similar in appearance. The adaxial surface is, in general, devoid of any ornamentation while the abaxial surface is well defined into stomatiferous and nonstomatiferous regions by strongly convex outer epidermal walls. All of the samples examined are hypostomatic, with sunken stomata subjacent to 4 to 6 subsidiary cells.

Whereas examination of leaf characters failed to reveal any morphological variation of taxonomical significance, what was revealed attests to the overall genetic similarity among the species of *Taxus*. Stomatal complexes, including guard cell shape and configuration, subsidiary cell number and arrangement, and suprastomatal cavities, appear stable and constant for the five species included in this study. Epidermal topography of both adaxial and abaxial surfaces, including cellular patternization, absence or presence of structures, and cellular configuration, likewise appear indistinguishably stable.

Data obtained from plastic epidermal imprints including stomate density and band width calculations may serve as viable taxonomical tools at the intraspecific level; however, they are ineffectual at the interspecific level due to variation and overlap. Counts obtained from plastic epidermal peels closely approximated counts made from SEM micrographs.
CHAPTER 2

POLLEN MORPHOLOGY

Literature Review

Pollen grains have been called the expression of the structural and functional evolution of plants (Nair, 1970), and in the words of R.P. Wodehouse (1959):

"Pollen grains are as much a part of the plant as the various organs upon which he (the taxonomist) has drawn to build his imaginative and surprisingly beautiful classification. But in this he has consistently ignored the pollen grains. In his rejection of them he has thrown away, perhaps, the richest part of his heritage, for in no other part of the plant are to be found packed in so small a space so many readily available phylogenetic characters."

The phylogenetic characters of which Wodehouse spoke have been revealed at a rate nearly commensurate to the rate at which microscopy has advanced, if for no other reason, because of their small size which ranges from less than five to greater than 200 microns (Faegri and Iverson, 1964).

Regardless of the century in which microscopy first became useful to palynological studies (for a complete review see Wodehouse, 1959), the current state of the art for both light and scanning electron microscopy (SEM) is now indispensable to detailed analysis of pollen grains.

Because resolving power and depth of field capabilities of SEM provide more information in three dimensional clarity than otherwise
possible with light microscopy, even at comparable magnifications, its application to pollen study is unanimously applauded (Grant, 1972; Heslop-Harrison, 1971; McGlone, 1978; Parthasarathy, 1970; Philbrook and Bogle, 1981).

The outer layer of the pollen wall to which this chapter is addressed is called the exine (Erdtman, 1957; Erdtman, 1969; Kapp, 1969; Faegri and Iverson, 1964; Nair, 1970; Wodehouse, 1959). The exine surface exhibits ornamentation in varying degrees, resulting in a highly complex pattern with high taxonomic specificity (Faegri and Iverson, 1964; Grant, 1972; Heslop-Harrison, 1971; Nair, 1970; Rosen, 1968; Stanley and Linskens, 1974).

Contributing to exine ornamentation are apertures, including their frequency, orientation, outline, and subunit composition, on which a systematic classification of pollen types has been soundly built (Erdtman, 1959; Erdtman, 1969; Faegri and Iverson; 1964, Wodehouse, 1959). In addition, ornamentation is determined by varying degrees of sculpturing. Sculpturing comprises the external geometric features without regard to their internal construction and is based upon various forms and arrangements of sculpturing elements (processes) which project beyond the outermost continuous layer of the exine (Erdtman, 1969; Faegri and Iverson, 1964; Kapp, 1969).

These two factors that contribute to ornamentation, together with pollen grain size, shape and polarity (ratio of the polar axis to the equatorial axis), are the criteria on which elaborate classification schemes have been built.
Historically, palynological data have been used to dispute (Argue, 1981; Feuer and Tomb, 1977; Grant, 1972; Mathewes, 1978) or confirm (Feuer and Kuijt, 1978; Gornall, 1977; Keely and Jones, 1977) phylogenetic relationships, and to correlate evolutionary and geographical factors to morphological features (Argue, 1981; Bolick, 1978; McGlone, 1978; Simpson and Skvarla, 1981; Tomb, 1975). Of equal interest, however, is the application of pollen morphology as a tool to aid in species and clonal identification.

Based upon differences in surface sculpture, Smit (1973) was able to identify three distinct pollen types in Eurasian species of Quercus, and correlated pollen type to deciduous or evergreen habit. Likewise, Philbrook and Bogle (1981) published that pollen from species of Acer native to the New England area fell into three distinct morphological pollen types.

Examination of fine structure in five species of Palm enabled Parthasarathy (1970) to distinguish amongst them whereas fine structure, in conjunction with pollen size, aided Fogle (1977) in the separation of fruit tree species including peach, nectarine, and apricot. In the same study, the author suggested that cultivars of tree fruit species could be distinguished based upon the number and types of apertures and, in a companion paper, Fogle (1977) presented evidence to substantiate his claim. Clones of peach, nectarine, European plum, and sweet cherry were separated on the basis of aperture number and size, and ridge width of exine sculpturing. He further noted that while pollen size varied within the clones, surface patterns remained constant.
Westwood and Chalice (1978) also reported clonal stability in exine topography in their examination of pear species. Whereas Maas (1977) was less successful in completely separating individual strawberry cultivars, he was able to distinguish four groups based upon exine ridge patterns.

Most recently, pollen from 8 ornamental crabapples was examined and exine topography and perforation type, size and frequency, enabled Martens and Fretz (1980) to separate all eight cultivars using a dichotomous key for the aforementioned characters. Moreover, Cargnello, et al. (1981) separated grape cultivars in their pollen analysis and additionally reported that external pollen morphology remained unchanged in their comparison of biotypes from different regions.
Materials and Methods

Pollen of 18 cultivars of 5 species as set forth in Method of Study, that had been labeled and stored in individual glass vials was sputter-coated with approximately 200A Platinum in a Polaron Equipment Limited SEM Coating Unit E5100, and subsequently examined on an International Scientific ISI-40 SEM operated at 30 kv, at a 20° tilt, and 8 mm working distance. Both coating and examination were performed in the Department of Plant Pathology EM Laboratory, OARDC, Wooster, Ohio.

After having scanned stub surfaces thoroughly to insure representative pollen grains were chosen, 4 random grains from replicate samples were examined and photographed. Pollen grains of abnormal shape or size were carefully excluded. Polaroid micrographs and negatives were collected from which quantitative data were compiled.

The widths of sculpturing elements (Fig. 10) were measured with a 7x magnifying ocular on 25 randomly selected elements in each of eight micrographs for each sample. Also, density counts per unit area were compiled for sculpturing elements using a clear acetate overlay with a marked field of reference. As elements were counted or measured, they were marked with an erasible pen in order to prevent counting or measuring an element more than once.

In order to assess the accuracy of measurement, polystyrene latex beads with a known diameter of .557 um (Dow Chemical Company, Midland,
Michigan) were mounted and photographed at the same tilt and working distance at which the pollen grains were examined. Replicate sets of twenty-five measurements of width were taken.

Data expressed below represent mean values from either measurements or counts and, where differences occur, means are declared significantly different by use of Duncan's Multiple Range Test at the 5% level.

Also included in the following discussion are micrographs and data from an earlier related study. Pollen samples from some of the same accessions collected in 1978 and 1979 were, after having been collected and mounted, coated with approximately 200A Gold on a Techniques Hummer III coating unit and examined on a Hitachi S-500 SEM in the Department of Botany, The Ohio State University, and will be identified accordingly.

Length measurement of whole pollen grains suspended in immersion oil were made at 400x magnification using an American Optical Phase-Star ONE-TEN light microscope with a 40x Plan Achro objective and a 10x ocular. Fifty pollen grains from each sample were measured at the point of maximum width and data are expressed as means with standard deviation (SD).

Descriptive terminology will follow Erdtman (1969).
Figure 10. *Taxus canadensis* pollen grain surface with sculpturing elements (arrow). Magnification bar equals 5 um.
Results and Discussion

In general, the pollen grains from the five species and their cultivars are irregularly shaped, appearing spheroidal to angular in outline (Fig. 11A). They are inaperturate, that is, there are no apparent pores or colpi (furrows), although it has been reported that T. brevifolia possesses a slightly bulging area where the exine is visibly thinner than the rest of the grain (Erdtman, 1965; Nair, 1965; Wodehouse, 1935). They do not remain united with other pollen grains at maturity but occur singly and therefore are referred to as monads. As reported by Hawker (1930), following the tetrad stage the individual spores separate early in microsporogenesis.

Size, expressed as the length of the longest axis, ranges from 18 to 29 μm with an average length of 22 for all samples.

According to the terminology proposed for angiosperm pollen, the sculpturing pattern is gemmate in which the sculpturing elements (protrusions) are at least in one dimension equal to or less than one micron, and the greatest diameter of the radial projection is equal to, or greater than the height of the element (Fig. 11B). The sculpturing elements are termed gemmae according to Erdtman (1969) because, in addition to their relative dimensions, they are not pointed and they are constricted at their lower parts (Fig. 11B). As will be discussed in Chapter 3, they are part of, and appear continuous with, the pollen wall.
Upon first inspection of Fig. 11A, it was suspected that the angularity and collapsed appearance of the pollen grain may have resulted from the vacuum \(5 \times 10^{-6}\) Torr imposed upon the grain while in the specimen chamber of the SEM. However, in a comparison light micrograph (Fig. 11 D,C), in which freshly harvested pollen was examined under no external constraints, it is clear that the shape is not an artifact of vacuum, but appears to be its shape \textit{in vivo}.

Size, expressed as length of the longest axis, was calculated rather than polarity indices because the components (polar axis, equatorial axis) could not be determined. The polar axis is an imaginary line through the proximal face (that part facing inwards at the tetrad stage) and the distal face (that part facing outwards at the tetrad stage). The equatorial axis is an imaginary plane running perpendicular to the polar axis midway between the distal and proximal faces. Based upon relations between the polar axis \((P)\) and the equatorial axis \((E)\) shape classes were suggested by Erdtman (1943).

The classes range from Perprolate in which \(P/E\) equals 2 (8:4) to Peroblate where \(P/E\) equals .50 (4:8) with intermediate classes and values between the extremes. Unless a triradiate scar is evident denoting the last place of contact between the four daughter-cells of a tetrad, it is impossible to distinguish proximal and distal poles in a single acolpate pollen grain. Pollen grains from \textit{T. floridana}, however, have been described as subprolate (Kapp, 1969) in which \(P/E = 1.33\) to 1.14 (8:6 - 8:7), suggesting that the longest axis in this case may coincide with the polar axis, if he was in fact able to identify the polar axis.
Figure 11. *Taxus* pollen at low and high magnification in which the
overall shape and detail are revealed. A) *T. cuspidata*
'Prostrata.' Magnification bar equals 5 um. B) *T. baccata*
'Aurea' sculpturing element detail (arrow). Magnification
bar equal 1 um. C) Light micrograph of thick sections of
*T. media* 'Brownii' stained with toluidine blue.
Magnification bar equals 25 um. D) Light micrograph of
unstained pollen of *T. media* 'Brownii' suspended in
immersion oil. Magnification bar equals 25 um.
Pollen grain sizes for the five species are presented in Table 6. *T. media*, for which the value was compiled from six cultivars, averaged the smallest size at 20.16 μm, whereas pollen grains of *T. canadensis* had the largest value at 25.65 μm (Table 6).

In Table 7, in which cultivars from *T. baccata* are compared, 'Nigra' is the only cultivar that differs appreciably from the others. Similarly, in Tables 8 and 9 in which size values for cultivars within *T. cuspidata* and *T. media*, respectively, are presented, there is little variation, perhaps, with the exception of 'Amherst' in Table 9.

Before being tempted to assign any taxonomical significance to the above mentioned examples, it should be noted that previously assigned dimensions of *Taxus* pollen varies greatly. For example, Kapp (1969) described *T. canadensis* and *T. floridana* pollen grains indistinguishable at "about" 28 μm. Wodehouse (1959) listed *T. brevifolia* as 23.9 to 26.8 μm in diameter, whereas Erdtman (1943) assigned values of 22 by 30 μm to the same species. Faegri and Iverson (1964) were more generous in requiring only that *Taxus* pollen be less than 40 μm.

Furthermore, although investigators using pollen size alone have been able to identify higher ploidy levels associated with larger size (Gornall, 1977; Mass, 1977; Yamaguchi, 1980), other studies have indicated that size dimensions are not reliable genetic characters in and of themselves (Bolick, 1978; Fogle, 1977; Fogle, 1977; Mathewes, 1978; Stanley and Linskins, 1974).
Table 4. Pollen diameter in *Taxus* species, expressed as length of longest axis.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Pollen Diameter (um) *</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. canadensis</em></td>
<td>25.65 ± 1.32</td>
</tr>
<tr>
<td><em>T. hunnewelliana</em></td>
<td>22.50 ± 1.26</td>
</tr>
<tr>
<td><em>T. cuspidata</em></td>
<td>21.60 ± 2.25</td>
</tr>
<tr>
<td><em>T. baccata</em></td>
<td>22.97 ± 4.56</td>
</tr>
<tr>
<td><em>T. media</em></td>
<td>20.16 ± 2.73</td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation
Table 5. Pollen diameter of cultivars within *T. baccata*, expressed as length of longest axis.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Pollen Diameter (um) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Aurea'</td>
<td>21.68 ± 1.00</td>
</tr>
<tr>
<td>'Dovastoniana'</td>
<td>19.55 ± 1.15</td>
</tr>
<tr>
<td>'Erecta'</td>
<td>20.98 ± .95</td>
</tr>
<tr>
<td>'Nigra'</td>
<td>29.68 ± 2.28</td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation
Table 6. Pollen diameter of cultivars within *T. cuspidata*, expressed as length of longest axis.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Pollen Diameter (um) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Adams'</td>
<td>20.65 ± 1.05</td>
</tr>
<tr>
<td>'Nana'</td>
<td>22.68 ± 1.43</td>
</tr>
<tr>
<td>'Hiti'</td>
<td>24.86 ± 1.43</td>
</tr>
<tr>
<td>'Prostrata'</td>
<td>22.73 ± 1.25</td>
</tr>
<tr>
<td>'Intermedia'</td>
<td>20.10 ± 1.04</td>
</tr>
<tr>
<td>'Thayerae'</td>
<td>18.58 ± .96</td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation
Table 7. Pollen diameter of cultivars within *T. media*, expressed as length of longest axis.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Pollen Diameter (um) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Amherst'</td>
<td>25.70 ± 1.71</td>
</tr>
<tr>
<td>'Brownii'</td>
<td>19.03 ± .92</td>
</tr>
<tr>
<td>'Burr'</td>
<td>18.73 ± .99</td>
</tr>
<tr>
<td>'Hatfield'</td>
<td>18.75 ± .91</td>
</tr>
<tr>
<td>'Ohio Globe'</td>
<td>19.28 ± .95</td>
</tr>
<tr>
<td>'Wellesleyana'</td>
<td>19.45 ± .95</td>
</tr>
</tbody>
</table>

*Mean ± Standard Deviation*
As mentioned previously, the ornamentation in *Taxus* is restricted to the presence of gemmae (a term referring to exine protrusions of certain dimension and form according to Erdtman, 1969). Graphically, the individual elements appear conglomerate, seeming to have subunits of their own (Fig. 12A), while some appear smooth and entire (Fig. 12B). No taxonomical significance, however, can be assigned to the occasional element surface variation because these differences were observed within the same species, and in some cases, cultivars. Possibly the smooth surfaces appear smooth due to lack of resolving power in isolated instances where specimens were improperly coated or impurities in the specimen chamber distorted the beam current, or the grains that differ may be coated unevenly with topetal debris.

Similarly, in several micrographs minute bumps are evident between the larger gemmae, whereas in others they are absent (Fig. 13 A,B). Such a surface is termed scabrous (Erdtman, 1969) and because it is apparent in many micrographs of the same species and cultivars, its occasional absence may be due to lack of resolution or focusing power in isolated instances, or differing coats of topetal debris. Comparative micrographs for each of the cultivars within their species are presented in Figures 14, 15, and 16.

In spite of the paucity of literature directed specifically towards *Taxus* pollen, Hawker (1930) conducted a developmental study concerning macrosporogenesis in *Taxus* in which she concluded that the pollen enters the free spore stage within the first three weeks in November. Within a temperate climate in which the average temperature between October and
April averaged 46° F, 46° F, 48° F, for the years 1977, 1978, and 1979, respectively, in Ohio, substantial morphogenetic activity subsequent to that time period would not be expected. Furthermore, it is well established that the major features of the sexine are established early in the tetrad stage and subsequent changes are mainly structural development of already existing features (Heslop-Harrison, 1971).

Therefore, the pollen grains collected from the expanded strobili are considered to be structurally mature on which valid taxonomic interpretations can be made.

Quantitative data for element diameter and density per unit are presented in Tables 8, 9, 10, and 11. In Table 8, in which species are compared, four of the species are distinguishable based upon element diameter and three are distinguishable based upon element density. It must be recalled, however, that the values for the species were compiled from a number of cultivars rather than from uncultivated species, and therefore, they may not truly reflect the species character.

As with leaf characters which were examined in Chapter 1, a comparison of species values to cultivar values illustrates overlap for both element size and density from one species to another. For example, in Table 9, *T. baccata* 'Erecta' with an element diameter of 0.48 um closely approaches the element diameter average for *T. canadensis* (Table 8). Likewise, *T. cuspidata* 'Hitii' with an element density of 10.89 (Table 9), falls into the range of *T. hunnewelliana* with an element
Figure 12. Detail of sculpturing elements in which two types are sometimes noticeable. A) Smooth surface of sculpturing elements of *T. canadensis*. B) Multiple sculpturing elements of *T. media* 'Brownii.' Magnification bar equals 1 um.
Figure 13. Two types of pollen surfaces were noted between the sculpturing elements. A) Minute projections (arrows) on the surface of *T. cuspidata* 'Prostrata'. B) Smooth surface between sculpturing elements of *T. baccata* 'Aurea.' Magnification bar equals 1 µm.
Figure 14. Electron micrographs of surface sculpturing elements from which quantitative size and density measurements were calculated. A) *T. canadensis*, B) *T. baccata* 'Dovastoniana,' C) *T. hunnewelliana*, D) *T. baccata* 'Erecta,' E) *T. baccata* 'Aurea,' F) *T. baccata* 'Nigra.' Magnification bar equals 2 um.
Figure 15. Electron micrographs of surface sculpturing elements of cultivars within *T. cuspidata*. A) 'Adams,' B) 'Prostrata,' C) 'Hiti,' D) 'Intermedia,' E) 'Nana,' F) 'Thayerae.' Magnification bar equals 2 um.
Figure 16. Electron micrographs of sculpturing elements of cultivars within *T. media*. A) 'Amherst,' B) 'Hatfield 23,' C) 'Brownii,' D) 'Ohio Globe,' E) 'Burr,' F) 'Wellesleyana.' Magnification bar equals 2 μm.
Table 8. Sculpturing element (gemmae) diameter and density for Taxus species.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Diameter (μm)</th>
<th>Density #/μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. canadensis</td>
<td>.51 a²</td>
<td>5.49 a</td>
</tr>
<tr>
<td>T. hunnewelliana</td>
<td>.33 b</td>
<td>10.79 b</td>
</tr>
<tr>
<td>T. baccata</td>
<td>.39 c</td>
<td>5.38 a</td>
</tr>
<tr>
<td>T. cuspidata</td>
<td>.35 b</td>
<td>8.02 c</td>
</tr>
<tr>
<td>T. media</td>
<td>.41 d</td>
<td>6.40 a</td>
</tr>
</tbody>
</table>

² Mean separation by Duncan's multiple range test, 5% level.
Table 9. Sculpturing element (gemmae) diameter and density for cultivars within T. baccata, T. cuspidata, and T. media.

<table>
<thead>
<tr>
<th>T. baccata</th>
<th>Diameter (um)</th>
<th>Density #/um^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Aurea'</td>
<td>.43 a</td>
<td>5.47 a</td>
</tr>
<tr>
<td>'Dovastoniana'</td>
<td>.31 b</td>
<td>4.81 b</td>
</tr>
<tr>
<td>'Erecta'</td>
<td>.48 c</td>
<td>5.35 a</td>
</tr>
<tr>
<td>'Nigra'</td>
<td>.34 d</td>
<td>5.88 c</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T. cuspidata</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>'Adams'</td>
<td>.30 a^2</td>
<td>6.26 a</td>
</tr>
<tr>
<td>'Hiti'</td>
<td>.33 b</td>
<td>10.79 b</td>
</tr>
<tr>
<td>'Nana'</td>
<td>.44 c</td>
<td>7.61 c</td>
</tr>
<tr>
<td>'Prostrata'</td>
<td>.37 d</td>
<td>8.25 c</td>
</tr>
<tr>
<td>'Intermedia'</td>
<td>.37 d</td>
<td>7.77 c</td>
</tr>
<tr>
<td>'Thayerae'</td>
<td>.28 a</td>
<td>7.44 c</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T. media</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>'Amherst'</td>
<td>.37 a^2</td>
<td>7.69 a</td>
</tr>
<tr>
<td>'Brownii'</td>
<td>.41 b</td>
<td>7.54 a</td>
</tr>
<tr>
<td>'Burr'</td>
<td>.41 b</td>
<td>7.68 a</td>
</tr>
<tr>
<td>'Hatfield 23'</td>
<td>.45 c</td>
<td>5.39 b</td>
</tr>
<tr>
<td>'Ohio Globe'</td>
<td>.46 c</td>
<td>5.23 b</td>
</tr>
<tr>
<td>'Wellesleyana'</td>
<td>.38 a</td>
<td>4.89 b</td>
</tr>
</tbody>
</table>

^2 Mean separation by Duncan's multiple range test, 5% level.
density of 10.79 (Table 8). Therefore, although statistical differences exist, the data in Table 8 are only indices and cannot be used as reliable taxonomical boundaries.

Interspecific comparisons of cultivars are set forth in Tables 9. For cultivars within T. baccata, all were separable based upon element diameter (Table 9). Density values on the other hand, only distinguished the cultivars into two groups.

In Table 9, 'Prostrata' and 'Intermedia' are the only two cultivars of T. cuspidata that cannot be separated from one another based upon both element size and element density. The remaining four cultivars are distinct in at least one of the two pollen characters (Table 9).

Cultivars within T. media were found to be the least well-defined by element characteristics (Table 9). Whereas three groups based upon element diameter, and two groups based upon element density are discernible, individual values for both characters lack variation (Table 9).

As it was pointed out in the Materials and Methods, pollen grains from some of the same accessions being used in this study were collected and examined for a related study in 1979. In Table 10 values for element diameter from the two independent studies are compared. Although there is some latitude, the values appear to be stable, particularly for T. canadensis, T. cuspidata 'Thayerae' and T. media 'Brownii'. This suggests that element diameter in Taxus pollen is
constant and in conjunction with other megamorphological features
may be serviceable in separating indistinguishable cultivars in *Taxus*.

To an extent, cultivars within *T. baccata* and *T. cuspidata* are
delimited by sculpturing element size and density, while only groups of
cultivars are discernible within *T. media*. Consistent with previous
reports, sculpturing element size has enabled other workers to
distinguish cultivars individually or by group (Cargnello, et al., 1981;
Fogle, 1977; Maas, 1977; Westwood and Chalice, 1978). Seldom, however,
has a single pollen character sufficed in differentiating taxa at any
one level. Because *Taxus* pollen grains are inaperturate (lacking pores
or furrows) an entire set of comparative features that may otherwise be
useful is lacking. Additionally, because the sculpturing elements in
all of the samples studied are gemmate and lack morphological variation,
their descriptive application to cultivar separation is of little value.

Because the only way of eliminating error in measurements performed
with scanning electron micrographs is to utilize an internal standard,
great scrutiny and care was employed in avoiding measuring an object
that was beyond the plane of focus, or distorted in any way.

In order to assess the accuracy of the technique by which the
sculpturing elements were measured, polystyrene latex beads of a known
diameter of .557 um were measured. The values, based upon 25 measure-
ments, varied only 6 to 7% from the actual diameter which is well within
the limits of biological research.
Table 10. Comparative values for element (gemmae) diameter for pollen collected and examined in 1979 and 1981.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>1979</th>
<th>1981</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. canadensis</td>
<td>.53</td>
<td>.51</td>
</tr>
<tr>
<td>T. baccata 'Dovastoniana'</td>
<td>.39</td>
<td>.31</td>
</tr>
<tr>
<td>T. cuspidata 'Prostrata'</td>
<td>.33</td>
<td>.37</td>
</tr>
<tr>
<td>T. cuspidata 'Thayerae'</td>
<td>.31</td>
<td>.28</td>
</tr>
<tr>
<td>T. media 'Brownii'</td>
<td>.37</td>
<td>.41</td>
</tr>
</tbody>
</table>
Table 11. Polystyrene latex beads: diameter measurements compiled from micrographs.

<table>
<thead>
<tr>
<th>Micrograph</th>
<th>Diameter (um)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>.59 ± .02</td>
</tr>
<tr>
<td>B</td>
<td>.60 ± .01</td>
</tr>
</tbody>
</table>

*Mean ± Standard Deviation.
Summary

Of the species and cultivars examined in this study, *Taxus* pollen is irregularly shaped and angular in outline, ranging in size from 18.58 to 29.68 μm. They are inaperturate, and the sculpturing type is gemmate. The surface between gemmae, in some cases, is scabrous. Overall pollen size, based upon length of the longest axis, is variable in some instances; however, it appears to be of no taxonomic significance.

Sculpturing element (gemma) diameter and density serve to delimit cultivars within *T. baccata* and *T. cuspidata*, however, separate only groups of cultivars within *T. media*. In comparing element size for pollen from two separate studies conducted two years apart, they appear stable.

Morphologically, *Taxus* pollen appear similar in their entirety and component sculpturing elements, precluding their application as a conclusive tool for cultivar separation.
The wall structure in pollen is unique among plant cell wall structures. Although it is sculptured with a great variety of patterns, there is a great degree of consistency in its organization (Esau, 1977). The wall of the pollen grain is essentially composed of two layers: the intine and exine (Erdtman, 1969; Faegri and Iverson, 1964; Kapp, 1969; Nair, 1970).

The intine, present in all pollen grains, immediately surrounds the living protoplasm (Faegri and Iverson, 1964). Chemically, the intine is partly cellulose with substantial amounts of pectic compounds, callose, and other polysaccharides (Kapp, 1969; Gorska-Brylass, 1968; Heslop-Harrison, 1971; Martens and Waterkeyn, 1961). Generally, the intine appears to be an homogeneous layer; however, in a study of ten species of *Pinus*, Martens and Waterkeyn (1961) detected two intine layers differing in their relative amounts of lipid and pectic compounds using soudan black aqueous stain and flourescence and phase contrast microscopy. Similarly, Freytag (1968) reported three layers of intine in *Viola tricolor* pollen, based upon their relative amount of pectic compounds. Heslop-Harrison and Knox (1971) suggested that the occasional layered appearance of the intine in transmission micrographs was due in part to imbedded proteins.
The exine, which is the outermost layer of the pollen wall, is extraordinarily resistant to chemical and biological decay (Stanley and Linskens, 1974). This layer is composed of a unique, highly unsaturated bipolymer called sporopollenin, which is thought to be composed of oxidized polymers of carotenoids or carotenoid esters (Brooks and Shaw, 1971).

The exine is generally distinctly layered into strata for which various authors have proposed elaborate terminologies (Erdtman, 1952; Erdtman, 1969; Faegri and Iverson, 1964; Nair, 1970; Praglowski and Punt, 1973). However, the bulk of information and descriptions are addressed to angiosperm pollen and, therefore, only the applicable counterparts to gymnosperm pollen in their simplest context will be mentioned lest they be confused.

The exine usually consists of two well-defined layers. The outermost layer which is usually sculptured is called the sexine and the inner layer, adjacent to and inferior to the sexine, is called the nexine (Erdtman, 1969). The nexine and sexine have been further divided into nexine 1, nexine 2, and bacula and tectum, respectively, for various angiosperm pollen types and, whereas subdivisions within gymnosperm wall layers may exist, these terms will be avoided for fear that their usage will suggest homogeneity for angiosperm and gymnosperm pollen features.

Differential aqueous staining, and the Lux Obscuritas technique with light microscopy, in which interpretations are made based upon diffraction images produced while downward focusing through the pollen
wall, are the methods by which Erdtman (1969) and his contemporaries delimited these layers. Currently, however, transmission electron microscopy has enabled investigators to refine or confirm earlier observations in pollen wall studies based upon differential electron densities detectable in the micrograph image (Meek, 1977).

Until the close of the 19th century, pollen morphology was studied in order to advance the knowledge of morphological botany (Nair, 1970). The application of pollen morphology since that time, however, is diverse and continues to increase in scope.

Taxonomically, wall architecture has served well to delimit familial boundaries (Feur and Kuijt, 1978; Dickenson, 1979; Nowicke, 1976) and elucidate intrafamilial relationships (Argue, 1981; Feuer and Tomb, 1977; Mathewes, 1978). Likewise, phylogenetic and evolutionary hypotheses have been proposed as a result of comparative pollen wall studies of major plant families (Bolick, 1978; McClone, 1978; Simpson and Skvarla, 1981; Tomb, 1975).

Developmental studies on the pollen wall have provided detailed accounts of the sequential stages in exine formation after meiosis (Heslop-Harrison, 1963; Echlin and Godwin, 1969), and have helped to determine that Ubisch bodies (agglomerations of sporopollenin) originate in the tapetum (Echlin and Godwin, 1968; Risueno et al., 1969). Ubisch bodies, which are spheroidal structures found in the anthers of many genera of angiosperms and some gymnosperms, are homologous to the exine and have been a subject of debate as to whether or not they play a role in exine formation (Echlin, 1971).
For transmission electron microscopy (TEM), pollen from *Taxus media* 'Brownii' was fixed for two hours in 5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 6.8. Following centrifugation, the pellets were rinsed six times, one hour each rinse in 0.1M sodium cacodylate. Following postfixation in osmium tetroxide (OsO₄) at 0 °C for one hour, the pellets were again rinsed in 0.1M sodium cacodylate four times, one hour in each rinse. After having been stained in uranyl acetate for thirty minutes and rinsed in 0.1M sodium cacodylate once, pollen residue was embedded in 7% agar and sectioned into 1-2 mm pieces. The agar sections were dehydrated in increasing concentrations of ethyl alcohol, (fifteen minutes per change in 25%, 50%, 70%, 90%, 95%, and three twenty minute changes in 100%) and subsequently embedded in plastic (Spurr, 1969). Thin sections were made with a diamond knife on a Sorvall Porter Blum MT2-B Ultramicrotome and collected on uncoated grids and poststained in 0.5% uranyl acetate and 0.2% lead citrate.

Electron microscope observations were made with a Philips Model 201 transmission electron microscope.

Thick sections of the embedded pollen (approximately 2 um) were made with a glass knife on a Pyramatome, stained with 1.0% toluidine blue in 2.5% sodium carbonate subsequently mounted on glass slides in immersion oil. Light microscopy and photography was performed on a Zeiss Photomic III photomicroscope.
Both light and transmission microscopy was performed at the Electron Microscopy Laboratory, Department of Plant Pathology, OARDC, Wooster, Ohio.

Descriptive terminology will follow Erdtman (1969) unless stated otherwise.
Results and Discussion

Thick sections of mature *T. media 'Brownii'* pollen that had been stained in toluidine blue are presented in Figure 17. Evident in the various section depths through the grains are the darkly stained cytoplasm and the distinct stratification of the outermost wall (Fig. 17). In agreement with what was reported in Chapter 2, the outline of the individual grains is irregular and angular in cross section (Fig. 17), further supporting the fact that the angularity of *Taxus* pollen is not imposed by the vacuum of the scanning electron microscope examination chamber. There are no discernible furrows or apertures and the outermost wall appears entire (Fig. 17).

In Figure 18A, pollen grains that had been placed in distilled water swelled and assumed a more spherical shape. Although still irregular in shape, after as little time as 1 minute in water, the angularity is lost (Fig. 18A). In addition to discernible layers in the outermost wall, the surfaces of some of the grains appear granular (Fig. 18A).

After various amounts of time ranging from less than one minute in some instances, to ten minutes, the exine ruptures irregularly exuding the uninucleate protoplasm (Fig. 18B). This agrees with a study by Takeuchi (1952) in which he reported similar rupturing of the exine, however, after as long as twenty minutes. In his investigation, Takeuchi further described the pollen wall of *T. cuspidata* after having
observed its germination, as being composed of an inner and outer layer and a so-called transparent layer which corresponds to the halo-like ring around the protoplasm in Figure 2B.

*Taxus* pollen was previously described in Chapter 2 as inaperturate based upon what the scanning electron micrographs revealed, which is in agreement with Erdtman (1943), and Faegri and Iverson (1964).

However, others (Nair, 1970; Ueno, 1959; Yamazaki and Takeoka, 1962) have reported a thin area in the exine of *Taxus* pollen which is called a leptome (Erdtman, 1969). The leptome or thin areas may play at least a physical role in the germination of *Taxus* pollen by yielding to the internal force imposed and thereby permitting the exine to rupture as previously observed.

The electron micrograph in Figure 19 displays an overall view of *Taxus media* 'Brownii' pollen in cross section. Occupying nearly sixty percent of the grain is the cytoplasm in which various cellular organelles are distributed (Fig. 19). Surrounding the cytoplasm is an electron translucent intine layer which averages approximately 2.3 um in thickness (Fig. 19). Adjacent to the intine are the outermost layers nexine and sexine including its component features, the perine and sculpturing processes, all of which will be discussed individually (Fig. 19).

In Figure 20, approximately one fourth of an entire pollen wall with its various components labeled accordingly, is presented. The outermost layer or sexine is composed primarily of the sculpturing
Figure 17. Thick sections (2 um) of *T. media* 'Brownii' pollen that had been imbedded in plastic and subsequently stained with toluidine blue reveal an overall angular outline, and layered outer wall. Cytoplasm (C), layered wall (WL). Magnification bar equal 25 um.
Figure 18. Untreated pollen grains from *T. media* 'Brownii' were placed in distilled water and after less than one minute swelled. A) The layered outer wall is discernible as are the surface processes. B) After several minutes the outer wall ruptures and the contents are released. Outer wall (OW), transparent layer (TP), cytoplasm (C). Magnification bar equals 25 um.
Figure 19. An ultrathin section through *T. media* 'Brownii' pollen gives an overview of the cell wall components and cytoplasmic organelles. Intine (IN). Magnification bar equals 20 μm.
Figure 20. The wall of T. media 'Brownii' in cross section is distinctly divided into layers of varying electron densities. Surface process (SP), perine (P), laminated layer (lam), inner layer (IL). Magnification bar equals 0.6 um.
processes (SP) or gemmae according to angiosperm terminology (Erdtman, 1969). They are irregularly distributed and range from approximately .15 to .48 um at their broadest axis in T. media 'Brownii.' Gullvag (1966), with transmission electron microscopy, reported that the "orbicules" of T. cuspidata pollen ranged in size from .25 to .30 um. Likewise, Yamazaki and Takeoka (1962), in their study of T. cuspidata, calculated from scanning electron micrographs a size range of .10 to .60 um for the surface processes.

Ueno (1959) and Gullvag (1966) further described the sexine as granular in obvious reference to the surface processes responsible for the sculpturing, however, referred to the individual elements as orbicules. Wodehouse (1959), at the level of light microscopy, described the exine of Taxus brevifolia as having closely packed granules.

Between the surface processes and still within the boundaries of the sexine, is a continuous yet amorphous electron dense layer which appears analagous to what has been described as tapetal debris in angiosperm pollen (Fig. 20). Various terms have been assigned to this loosely attached layer including tryphine (Erdtman, 1969; Echlin and Godwin, 1969), and Pollenkitt (Heslop-Harrison, 1968; Heslop-Harrison and Dickenson, 1969). Although Echlin (1971) differentiates Pollenkitt from tryphine based upon chemical composition, a more general term, perine, has been used to describe the surface coating of pollen grains in general (Heslop-Harrison, 1971) and gymnosperm pollen grains in particular (Ueno, 1959; Ueno, 1960).
The surface coatings in angiosperms, composed of various lipid and carotenoid pigments, are thought to play a role in entomophylous (insect dispersed) pollination because of the yellow color and odor they impart to the grain (Heslop-Harrison, 1968). Nevertheless, Taxus pollination is primarily anemophylous (wind dispersed) and therefore the function of its coating as an attractant remains a moot question.

The outer coating or perine does not appear to be uniformly distributed in thickness, nor does it appear densely compacted (Fig. 20). Its average thickness is approximately 0.12 um.

The sculpturing processes appear continuous with the pollen wall or, in some instances, appear slightly discontinuous (Fig. 21). Gullvag (1966) similarly reported that some of the sculpturing processes in T. baccata looked as if they were more deeply "rooted" in the wall than others.

This apparent discontinuity may be due to the fact that orbicules that appear independent of the wall are imbedded in the plastic section behind those that appear continuous and, therefore, their juncture with the wall is not within the plane of vision.

Also noticeable in Figure 21 is the outline of secondary sculpturing (SC) upon the orbicules. As reported in Chapter 2, scanning electron micrographs revealed that sculpturing elements on the pollen surface possessed a topography of their own which, in Figure 21, is discernible in cross section.

Below the sexine is a distinctly laminated layer which is termed the nexine (Fig. 20). The laminae, although not perfectly concentric,
Figure 21. The sculpturing processes of the pollen wall in *T. media* 'Brownii' are either in close proximity or slightly above the underlying nexine. Secondary sculpturing (SC), laminated layer (LAM), Inner Layer (IL), Perine (p), intine (IN). Magnification bar equals 0.6 µm.
appear continuous and follow the perimeter of the outermost wall (Fig. 20). Gullvag (1966) in his description of the pollen wall of *T. cuspidata* also called attention to an inner laminated layer and hypothesized that the laminae were due to different oxidation states of the sporopollenin.

Ueno (1959) described the nexine in *Taxus cuspidata* as laminated with 5 to 7 layers approximately .05 um in width. In Figure 21 the layers appear even more distinct and, similar to Ueno's (1959) calculations for *T. cuspidata*, range from .03 to .06 um in width.

An inner layer (IL) within the nexine can be distinguished which is similar in electron density to the perine (Fig. 21). Subdivisions within the nexine are recognized by Erdtman (1969) and others (Faegri and Iverson, 1964; Nair, 1970); however, the specific terms nexine-2 or endexine, depending upon the author, owe their origin to angiosperm pollen terminology. Ueno (1959), Gullvag (1966), and Takeuchi (1952), however, made no reference to subdivisions within the nexine of *Taxus cuspidata*.

The boundary between the laminated layer and the inner layer appears uniform, while the boundary between the inner layer and the intine is irregularly invaginated (Fig. 22). Whether or not this is an artifact of fixation and sectioning is not known, although this phenomenon was present in all of the micrographs examined.

The intine, as delimited in Figure 19, is situated between the inner layer of the nexine and the cytoplasm. This corresponds to its
Figure 22. The nexine layer of *T. media* 'Brownii' is clearly divided into a laminated and inner layer. Invagination (INV), intine (IN), Inner layer (IL). Magnification bar equals 0.6 um.
position as noted under light microscopy. The intine is partially comprised of callose (Gorska-Brylass, 1968; Stanley and Linskens, 1974), and callose turns cobalt blue when stained with resorcinol blue (Eschrich and Currier, 1964). Under a light microscope, a blue ring could be clearly seen within the exine and outside of the cytoplasm, of pollen grains that had been stained with resorcinol blue. The intine is pectocellulosic in composition (Martens and Waterkeyn, 1961) and appears electron transluscent, and microfibrilar in texture in transmission electron micrographs, which is in agreement with previous descriptions (Rowley, 1959; Vasil and Aldrich, 1971). Likewise, variation in electron density, as seen in Figure 19, suggests lamellation and ribboning throughout the intine as reported previously (Freytag, 1968; Martens and Waterkeyn, 1961; Rowley, 1959; Vasil and Aldrich, 1971). These same authors hypothesized that pectic, lipid and protein compounds were responsible for the lamellation due to their unequal distribution throughout an otherwise homogeneous cellulosic layer.

Figure 23 illustrates what previous investigators have referred to as Ubisch (UB) bodies in *Helleborus* and *Allium* (Echlin and Godwin, 1968; Risueno et al., 1969). Ubisch bodies are spheroidal structures occurring in large numbers on the walls of tapetal cells and released into the microsporangia upon autolysis (Echlin and Godwin, 1968; Echlin, 1971; Heslop-Harrison and Dickenson, 1969). Characteristically, they are delimited by a unit membrane (Echlin, 1971) and this membrane is discernible in Figure 23 (arrow). Additionally, they sometimes exhibit surface processes of their own which may or may not resemble surface
processes of the pollen grain (Echlin, 1971). For Taxus the sculpturing of the Ubisch body is similar to the sculpturing of the pollen orbicules and in some respects appears to be nothing more than detached orbicules (Fig. 23.)

Because Ubisch bodies consist of sporopollenin, the main constituent of a mature exine, early hypotheses considered that Ubisch bodies represented a transport mechanism for sporopollenin (Risueno, et al., 1969); however, others report that there is no evidence for remobilization of sporopollenin once it is deposited in a particular site (Echlin, 1971; Heslop-Harrison and Dickenson, 1969).

As pollen is collected from the male strobili, debris including tapetal tissue and Ubisch bodies are more likely collected also. Consequently, if the Ubisch bodies are in contact with the pollen wall, it is likely that they, too, were imbedded in plastic, explaining their presence in Figure 23. This is plausible since Banerje and Barghoorn (1971) reported that Ubisch bodies are attached to the spinules of pollen of certain grasses. Similarly, Yamazaki and Takeoka (1962) noted dense particles that adhered to the surface of T. cuspidata for which they offered no explanation other than that there were electron dense particles associated with the pollen surface.
Figure 23. Debris such as Ubisch bodies are often in close proximity to the pollen grains and consequently become embedded in sectioning. Ubisch bodies (UB), sculpturing (SC), membrane (m). Magnification bar equals 0.3 um.
Summary

The wall of *Taxus media* 'Brownii' pollen appears inaperturate; however, may possess a thin area (leptome) at which point it ruptures when immersed in water.

The wall is divided into two layers: an outermost sculptural sexine, and inner nexine.

The sexine is composed of surface processes of various sizes ranging from .10 to .60 um at their widest axis. They are unevenly distributed over the wall and are sculptured irregularly. For the most part they are in close proximity to the underlying nexine.

Within the boundaries of the sexine is an amorphous electron dense layer which is continuous between the surface processes which is called the perine. This coating, which has an average thickness of approximately .12 um, may be analogous to what other authors have termed tryphine or Pollenkitt in angiosperm pollen grains.

The inner layer, called the nexine, is composed of two distinct strata. A laminated layer directly below the sexine is delimited by 5 to 7 lamella that are circumferential and appear continuous. A second layer adjacent to the intine is detectable by its differential electron density. It comprises approximately 20% of the nexine and its boundary adjacent to the intine is invaginated. The average thickness of the total nexine is approximately .33 um.
The intine, while not appearing distinctly stratified, exhibits ribboning and lamellation, and its texture is microfibrilar. It stains cobalt blue in response to resorcinal blue, indicating the presence of callose.
CONCLUSIONS

The ability to distinguish among cultivars within economically important genera of ornamental plants for which conventional means of separation are inadequate, is of significant import to the horticultural community. Numerous cultivated varieties have been patented and introduced in recent years in response to a demand imposed by smaller landscapes and urban environments for which plants that exhibit restricted growth habits are required. In many cases, plants that eventually will exhibit excurrent, columnar, or prostrate growth habits are virtually indistinguishable in their juvenile stage of growth. Such is the case for the genus *Taxus* and its many cultivars for which, with the exception of the variegated foliage types, classification has been based solely upon growth habit.

Rather than a phylogenetic problem, the enigma with which nurserymen, plant patent officials, and the landscape industry as a whole are confronted is a challenge to explore systems by which information can be communicated.

The information to be communicated is the true identity of the plant in question and the classificatory system is based upon characters or distinctive marks by which the identity of the total plant can be summarized. Because the distinguishing characters with which we hope to identify and distinguish are not always manifest, we select from those that are available, or become available as a result of inquiry and investigation.
Within this context, it was the aim of this study to select and describe characters that have precedents in previous taxonomical studies and assess their communicative value to cultivar separation within the genus *Taxus*.

Because gross vegetative characters of *Taxus* that are measurable, countable or otherwise capable of being described, have failed to delimit taxonomical boundaries below the species level, features observable only with the electron microscope such as leaf surface and pollen wall features were selected.

As they were discussed separately in Chapters 1 and 2, the leaf surface characters, including stomate band width and stomate density, and pollen wall characters, including sculpturing element size and density, in and of themselves, were moderately successful in some instances in enabling one to separate the cultivars based upon the information they provided.

Synergetically, however, when all four characters were compared, all eighteen species and cultivars were separable from one another, fifteen of which were separable by no less than two characters, and seven of which were separable by three or four characters.

The following scheme employs all of the above mentioned parameters, excluding pollen grain diameter, and represents one possibility in categorizing members of a small group of cultivars or species. Within each range, conservative limitations were imposed upon each selection and each separation was within the mean separation values at the 5% level.
Figure 24. Schematic classification system for separating cultivars based upon stomate density, stomatal band width, pollen element size, pollen element density.
STOMATE DENSITY <14,000

T. hunnewelliana, T.m. 'Ohio Globe', 'Brownii', 'Amherst', T. baccata 'Erecta'

STOMATAL BAND WIDTH

>500um

T. hunnewelliana, T.m. 'Ohio Globe', 'Amherst'

≤500um

T. b. 'Erecta'

T.m. 'Brownii'

POLLEN ELEMENT DENSITY

>6

T. hunnewelliana

T.m. 'Amherst'

T.m. 'Ohio Globe'

≤6

T.m. 'Brownii'

T.b. 'Erecta'

POLLEN ELEMENT SIZE

>35um

T.m. 'Amherst'

T. hunnewelliana

≤35um
STOMATE DENSITY 14,000 - 20,000

T. canadensis, T. baccata 'Aurea', 'Dovastoniana',
T. cuspidata 'Adams', T. media 'Wellesleyana', 'Burr',
'Hatfield 23', T. cuspidata 'Intermedia'

STOMATAL BAND WIDTH

>500um

T.m. 'Wellesleyana'
T.m. 'Burr'

<500um

T.c. 'Intermedia', 'Adams'
T.b. 'Aurea', 'Dovastoniana'
T.m. 'Hatfield 23', T. canadensis

POLLEN ELEMENT DENSITY

>6

T.m. 'Burr'
T.m. 'Wellesleyana'

<6

T.c. 'Intermedia', 'Adams'
T.b. 'Aurea', 'Dovastoniana'
T.m. 'Hatfield 23', T. canadensis

ELEMENT SIZE

>.35um

T.c. 'Intermedia'
T.c. 'Adams'
T.b. 'Dovastoniana'
T. canadensis

<.35um

T.b. 'Aurea'
T.m. 'Hatfield 23'
STOMATE DENSITY >20,000

*T. cuspidata* 'Prostrata', 'Hitl', 'Nana', 'Thayerae',
* T. baccata* 'Nigra'

STOMATAL BAND WIDTH

- >500um
  - *T.c.* 'Prostrata'
  - *T.c.* 'Hitl'

- <500um
  - *T.b.* 'Nigra'
  - *T.c.* 'Nana', 'Thayerae'

POLLEN ELEMENT DENSITY

- >6
  - *T.c.* 'Prostrata'
  - *T.c.* 'Hitl'

- <6
  - *T.c.* 'Nana'
  - *T.c.* 'Thayerae'

POLLEN ELEMENT SIZE

- >35um
  - *T.c.* 'Prostrata'
  - *T.c.* 'Hitl'

- <35um
  - *T.c.* 'Nana'
  - *T.c.* 'Thayerae'
As plants were segregated at each level, they were grouped indiscriminately without regard to species; however, at successive levels, fell into species groups in some instances. Within this scheme, the only cultivar that did not separate from the others was *Taxus baccata* 'Aurea', a name given to cover several golden-foliaged forms, which is commonly propagated by seed in order to avoid plagiotropic growth. This perhaps suggests that other than exhibiting yellow foliage, 'Aurea' may be more closely related to other cultivars than otherwise suspected.

Although, ideally, classification should be made on an understanding of all characters, such a task is simply impractical if not impossible. Therefore, several characters diagnostic for separating two taxa are empirically determined and eventually are employed as useful features in the description and delimitation of groups of plants.

Whereas pollen and leaf surface features have shown to be viable characters for the separation of species and cultivars within the scope of this study, their ultimate value and utility can only be confirmed through future inquiry and scrutiny.
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