Mycoscience 37: 91–98, 1996

Biology and life cycle of *Atelocauda koae*, an unusual demicyclic rust

Wan-Quan Chen[1,2], Donald E. Gardner [1] and David T. Webb[1]

[1] Department of Botany, University of Hawaii at Manoa, Honolulu, Hawaii 96822, U.S.A.
[2] Institute of Plant Protection, Chinese Academy of Agricultural Science, West Yuan Ming Yuan Road, Beijing 100094, China

Accepted for publication 17 January 1996

***Atelocauda koae*, a rust of the native Hawaiian *Acacia koa*, is considered as a demicyclic species, having spermogonial, aecial, and telial states, but is unusual in production of aeciospores simultaneously with teliospores rather than consecutively. Host inoculation with spores of each state separately confirmed that the life cycle was perpetuated by the telial state, but the aeciospores, while capable of germination and stomal penetration, did not produce detectable infection. This rust therefore behaves as a microcyclic species, and appears to be in evolutionary transition toward this reduced state. Teliospores produced vestigial, permanently attached basidiospores which germinated to produce infective hyphae. The hyphae entered the host either through stomata or penetrated the epidermis directly, with the latter method being more common. Unusual nuclear behavior associated with teliospore germination, in which meiosis occurs in more than one diploid nucleus was observed, in confirmation of an earlier study.**

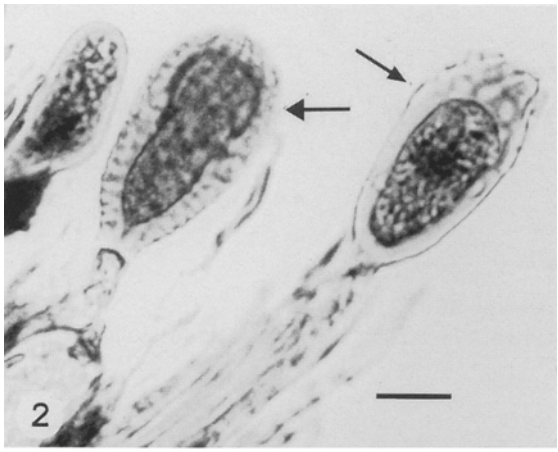
Key Words——*Acacia koa*; *Atelocauda koae*; fluorochrome staining; native Hawaiian fungi; teliospore germination.

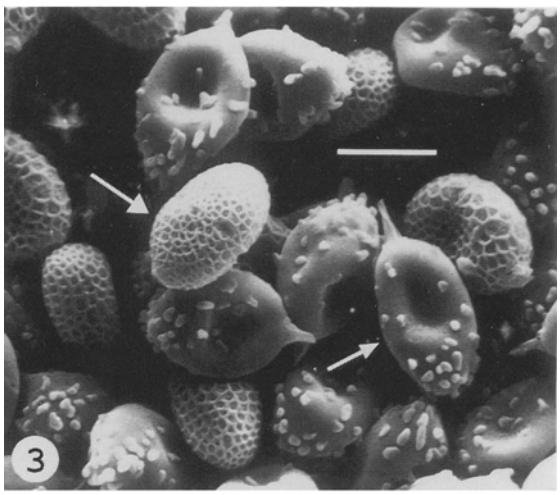
Atelocauda koae (Arthur) Cummins & Hiratsuka is one of five closely allied rust fungi which occur on *Acacia koa* Gray (koa) (Cummins and Hiratsuka, 1983; Hodges and Gardner, 1984). As an endemic species, this host occupies a critically important ecological role as the dominant overstory species of higher elevation forests throughout the Hawaiian Islands. *Acacia koa* is also highly valued in Hawaiian cultural practices and is commercially prized for the quality of its timber (Whitesell, 1990).

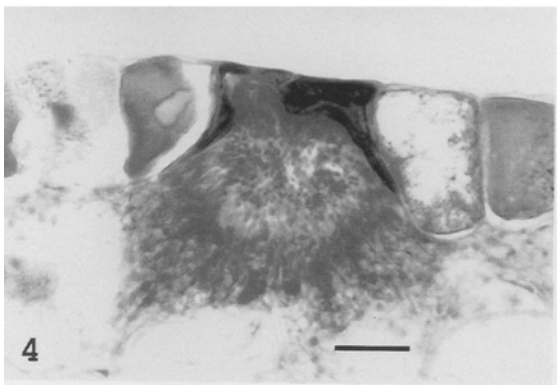
The genus *Atelocauda*, considered to have strong affinities to *Uromycladium* on *Acacia* spp. in Australia, is characterized by distinct, reticulately marked spores (Gardner and Hodges, 1985). The genus was revived in 1983 to accommodate the *Acacia* rusts of the Pacific, previously included in *Uromyces*, in an effort to more accurately reflect their true affinities (Cummins and Hiratsuka, 1983). However, the question of the correct taxonomic placement of these rusts remains open.

Infection by *Atelocauda koae* appears limited to young tissue of the leaf, phyllode, or stem, and can cause distortion of shoots, resulting in a deformed stem (Hodges and Gardner, 1984) (Fig. 1). The original description of *A. koae* (as *Uromyces koae* Arthur) mistakenly combined the life cycles of two currently-recognized species of *Atelocauda*, with *A. koae* as the telial state, and the aecial state of *A. digitata* (Wint.) Cumm. & Y. Hiratsuka (as *U. digitatus* Wint.) as the uredinial state (Stevens, 1925). However, Hodges and Gardner (1984) later described *A. koae* as a demicyclic species distinct from *A. digitata*, with telia, uredinoid aecia, and spermogonia (Figs. 2–4). Whereas the spore states of a fully functional rust life cycle are produced consecutively, the aecia and telia of *A. koae* occur simultaneously, with ae-

Fig. 1. Shoots of *Acacia koa* naturally-infected with *Atelocauda koae*.







Figs. 2–4. Spore states of *Atelocauda koae*. 2. Young aeciospore (large arrow) developing simultaneously with a teliospore (small arrow). 3. Scanning electron micrograph of a typical mixture of aeciospores (large arrow) and teliospores (small arrow). 4. Cross section of a spermogonium. Scale lines = 10 μm in Fig. 2; 20 μm in Figs. 3, 4.

ciospores consistently intermixed with teliospores (Figs. 2, 3), leading to questions concerning the relative roles of each of these spore states in the life cycle.

Earlier, Gardner (1981) reported unusual teliospore germination and nuclear behavior in *A. koae*, noting the production of branched germ tubes of teliospores, each with a diploid nucleus capable of meiosis, but normal deciduous basidiospores were absent. These observations were confirmed and supplemented in the present study through use of a DNA-specific fluorochrome (DAPI) for nuclear observation. The germ tube "branches" were recognized later as elongate germ tubes arising from vesicle-like swellings representing modified, non-deciduous basidiospores (Gardner, 1988). These germ tubes were presumed functional in reinfecting the host and completing the disease cycle, but direct observations were lacking. The present study was undertaken in part to confirm this function and to determine the mechanism of reinfection. Information on the influence of temperature and light regimes on teliospore germination also was provided in an effort to further understand the biology of this unusual rust.

Materials and Methods

Teliospore germination Infected host tissue bearing freshly sporulating rust was collected in Hawaii Volcanoes National Park, Hawaii, USA. Teliospores from each of three collection sites were floated on water droplets on microslides placed on moist filter paper in Petri dishes. The spores, in two sets of dishes, one covered with aluminum foil to exclude light, and the other exposed to continuous illumination at 5,000–7,000 lx, were incubated at 8–10 ,15–16, 20–21, 23–24, or 30–31 °C. To determine the onset and rate of germination, spore samples of each treatment were observed microscopically at 2-h intervals until germination began. Overall percentage of germination was determined after 24-h incubation. To further assess physical factors influencing germination, fresh teliospores were dusted onto dry slides and placed in Petri dishes with wet filter paper and incubated at 23–24 °C for 24 h. Other teliospores were dusted on the surface of 1.5% water agar and similarly incubated.

Nuclear staining Teliospores at progressive stages of germination were dried for 2 h at 45 °C on a slide warmer, or at room temperature (22–23 °C) for 12 h, and fixed in ethanol/acetic acid (3 : 1) for 15 min. A 1.0 mg/ml stock solution of the DNA-specific fluorochrome 4, 6-diamidino-2-phenylindole (DAPI) in distilled water which had been stored in the dark at 4 °C was diluted with McIlvaine's buffer (0.1 M citric acid [$H_3C_6H_5O_7$]/0.2 M Na_2HPO_4 ; 1 : 4.04) (pH 6.98) to make a 0.5 μg/ml solution, with which teliospores were stained for 15 min at 37 °C. The spores were washed before staining for 15 min, and after for 7 min, in McIlvaine's buffer. A drop of undiluted glycerol was placed on the stained spores, and a cover slip was added. For comparison, some teliospores were stained with HCl-Giemsa as previously described (Gardner, 1981). Although some teliospores were lost from the slides during the above procedures, enough remained affixed to provide adequate material for observation.

Staining of hyphal septa Teliospores which had germinated and dried on microscope slides were fixed with Singleton's fixative (acetic acid : lactic acid : absolute ethanol, 1 : 1 : 6) or FAA (acetic acid : formaldehyde : ethanol : water; 1 : 1 : 10 : 7) for 10 min, then stained

with Calcofluor White M2R ($C_{40}H_{42}N_{12}O_{10}S_2Na_2$) (Sigma) as follows: 95, 75, and 50% ethanol, 1-2 min each; distilled water successively through 2-3 staining jars, 5 min total; Tris-HCl (0.1 M, pH 8.3), 30 min; 0.1% Calcofluor White in Tris-HCl, 5 min; distilled water, 2-3 jars, 5 min total; 25% glycerol, 30 min. The tissue was mounted on a microslide in lactophenol and covered with a cover slip.

Stained material was observed with a Zeiss microscope equipped for epifluorescence with a 100 W DC mercury illuminator and a Zeiss 487702 filter combination. Photographs were taken with a Zeiss C35 camera and Ektachrome 160 or 200 slide film. Images were digitized on a ROM compact disk and prepared for publication with the Adobe Photoshop® (Adobe Systems Inc.) illustration production program.

Susceptibility to infection relative to host tissue age Koa plants grown from seed in 10 cm plastic pots were arranged in three groups representing plants 2 wk, 2 mo, and 6 mo old at the time of inoculation. Leaves were sprayed with a suspension of a freshly collected teliospore/aeciospore mixture supplemented with 0.05% Tween 20 (Baker Chemical Co.) to facilitate spore dispersion. Inoculated plants were incubated at 22°C at approximately 100% relative humidity for 48 h, then transferred to a lighted chamber (5,000-7,000 lx) and maintained at 20°C. After 20 d, leaflets of the three originally-produced pinnate leaves of each plant were examined for rust pustules.

For observation of stomata, leaves of the above ages were fixed and cleared in liquified phenol/lactic acid/chloral hydrate (1 : 1 : 2) for 24-48 h and observed with a light microscope. Six leaves of each age group were selected, and stomata in five microscope fields for each leaf were counted.

Colonization of internal tissue True leaves of the above three age groups were inoculated by spraying with an aeciospore/teliospore suspension as described. Inoculated plants were incubated at 22°C for 48 h at approximately 100% relative humidity, then moved into a chamber at 20°C supplemented with light at 5,000-7,000 lx. After 48 or 96 h, leaves were removed and cleared and stained with Calcofluor White M2R (Kang et al., 1993). The tissue was prepared as follows: 1) bleached in 99.9% methanol/99.9% chloroform (1 : 2; v : v) for 6 h; 2) fixed by boiling in lactophenol/95% ethanol (1 : 2; v : v) for 1.5-2.0 min followed by standing in the solution overnight (12 h); 3) Washed twice, 5 min each, in 50% ethanol, then washed twice, 5 min each, in distilled water; 4) cleared in 0.5 M NaOH for 10 min; 5) washed in distilled water twice, 5 min each time; 6) buffered in 0.1 M Tris-HCl buffer (pH 8.27) for 30 min; 7) stained with 0.1% Calcofluor White in the above buffer for 5 min; 8) washed in distilled water twice, 5 min each; 9) soaked in 25% glycerol for 30 min. The stained tissue was floated on a microscope slide in lactophenol and a cover glass was applied.

Tissue sectioning and staining For observation of infective structures, intact pre- and post-sporulating telial/aecial pustules on leaf or phyllode tissue were fixed with antifreeze fixative solution (10% dimethyl sulphoxide/4% paraformaldehyde/1% Tween 20/0.05 M sodium cacodylate (pH 7.2) at 20-25 lb. per square inch in a vacuum pump overnight. Tissue sections (0.3 × 0.5 cm) were embedded in Tissue-Tek® (Miles, Inc.) on a metal block at −20°C. Frozen tissue was sectioned at 10-16 μm with a Reichert-Jung Cryocut 1800 freezing microtome. Sections were mounted with Haupt's adhesive (Jensen, 1962), dried on a slide warmer at 35°C for 1 h, and then hydrated in 95, 75, and 50% ethanol for 1 min in each solution. Sections were washed twice with distilled water, processed through 0.1 M Tris-HCl buffer and stained with Calcofluor White as described above.

Stained tissue sections were examined with the Zeiss fluorescence microscope equipped as described above. Photographs were prepared for publication with the above-described illustration production program. The scanning electron micrograph (Fig. 3) was photographed by the standard procedures described by Gardner and Hodges (1985), after which the image was scanned to a diskette and processed with the illustration program.

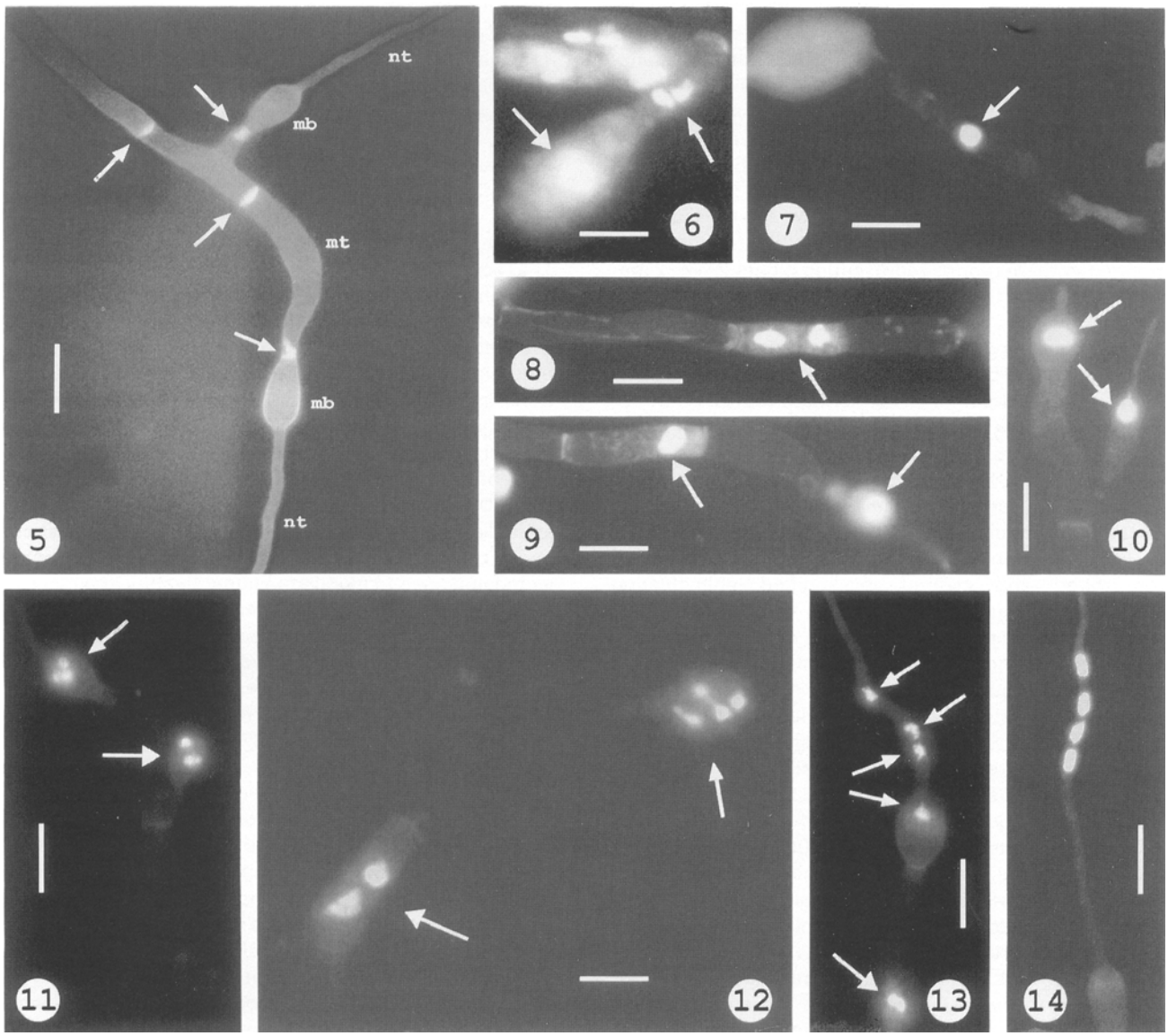
Serial inoculation Newly expanding leaves of young koa plants were inoculated by spraying with a suspension of a fresh teliospore/aeciospore mixture collected from field-infected plants. Inoculated plants were incubated in a 20°C chamber at 90% relative humidity and constant light at 5,000-7,000 lx. After 20 d, teliospores and aeciospores produced on resulting pustules were collected and used to inoculate young, healthy koa plants.

Separation of teliospores from aeciospores To determine the individual roles of teliospores and aeciospores in the life cycle of *A. koae*, it was necessary to inoculate with each type separately. Fresh spore mixtures collected from infected plants in the field were suspended in liquified 1% water agar at 35°C. Glass microscope slides were dipped in the suspension, coating them with a thin layer, which quickly solidified. Single, well-isolated spores were located microscopically under low power and manually removed from the slides with a capillary needle. Spores were transferred to pre-determined inoculation sites on young expanding leaves of healthy koa plants grown from seed in the greenhouse. Each plant was inoculated with either teliospores or aeciospores, but not both, with a concentration of 50-100 spores per inoculation site. This procedure was replicated 10 times. Inoculated plants were incubated for 20 d, following which symptom development was noted and some of the inoculated leaves were removed, cleared, and stained with Calcofluor White for examination of internal hyphae as described above. This procedure was repeated for three successive generations of spores resulting from infections produced by the inoculations.

Results

Influence of light and temperature on teliospore germination Germ tubes usually began to emerge from freshly collected teliospores after 4-8 h of incubation, depending

on the temperature and light conditions. Optimum germination was obtained at 23-24°C, with the minimum limit 8-10°C and maximum near 30-31°C, at which temperature trace germination occurred. At 20-24°C, germ tubes began to emerge after approximately 4 h of incubation, regardless of the lighting conditions. At 15-16°C, onset of germination was prolonged for 2 h (to 6 h) under continuous light, and for 4 h (to 8 h) under continuous darkness. Similar influences of light or darkness on incubation period at other temperature regimes were not evident. Significant effects of light and darkness on overall teliospore germination occurred, with continuous exposure to light resulting in higher percentages throughout the temperature regimes as compared



Figs. 5-14. *Atelocauda koae*. Teliospore germination and nuclear behavior. 5. Teliospore germ tube, including the main tube (mt) with Calcofluor White-stained septa (arrows) delimiting the region of the "branch" origin and the nondeciduous modified basidiospores (mb) of both the "branch" and the main tube. Narrow tubes (nt) arise from both modified basidiospores. 6. DAPI-stained diploid immature teliospore (large arrow) borne on a binucleate pedicel (small arrow). 7. Germinating teliospore with a diploid nucleus (arrow) migrating into the germ tube. 8. Septate region of the germ tube (arrow) delimiting two diploid nuclei associated with increased fluorescence of the cytoplasm. 9. Germ tube with one diploid nucleus remaining in the septate region (large arrow) and the other having migrated into the terminal modified basidiospore (small arrow). 10. Germ tube in which a diploid nucleus has migrated into the modified basidiospores of both the main tube (small arrow) and the "branch" (large arrow). 11. Meiosis in progress in the modified basidiospores of both the main tube (small arrow), and the "branch" (large arrow). 12. An unbranched germ tube with two recently divided diploid nuclei (large arrow) and meiotic division of a third nucleus nearing completion in the modified basidiospore (small arrow). 13. A series of four post-meiotic nuclei (small arrows) migrating from the modified basidiospore of the main tube into the narrow tube. A diploid nucleus (large arrow) is also in the "branch". 14. Migration of the four post-meiotic nuclei toward the end of a narrow "branch" tube. Scale lines = 20 μm in Figs. 5, 7-11, 13, 14; 10 μm in Figs. 6, 12.

Table 1. Influence of temperature and light on *A. koae* teliospore germination.

Temperature (°C)	Germination percentage[a]							
	24 h Continuous light[b]				24 h Continuous dark			
	I[c]	II	III	Means	I	II	III	Means
8–10	0	0	0	0	0	0	0	0
15–16	13	16	16	15	0.6	1	2	1
20–21	30	26	49	35	16	17	23	19
23–24	45	39	50	45	39	15	36	30
30–31	0	0	2	0.5	0	0	0	0

a) A total of 300–500 teliospores from five fields on each slide was observed.
b) 4,000–6,000 lx.
c) I, II, III represent teliospores from different collection sites.

to those of dark-exposed spores as summarized in Table 1.

Teliospores collected from different sites and on different dates from each other showed some inconsistency in germination percentages (Table 1), which probably resulted from variable internal physiological factors, such as age or spore maturity. Germination was more consistent among teliospores produced as a result of inoculation in the laboratory, but did not occur under any temperature or light regime in which teliospores were incubated for 24 h under high humidity but not in contact with water or agar surface.

Events of teliospore germination The unusual morphology of the germ tube noted previously (Gardner, 1981) was found consistently in the current study in which several hundred germinating teliospores were observed. The main tube usually lengthened to (126)-150-(230) μm and widened to (6)-7-(9)μm. The middle section of the tube became delimited with 1–3, but usually two, prominent septa forming at a midlength position for which Calcofluor White stain demonstrated affinity (Fig. 5). A fusiform to subglobose vesicle-like swelling, probably representing a modified basidiospore (Gardner, 1988), formed and was subtended by a septum. This structure germinated in place to form a lengthy, distinctly narrower tube measuring (122)-157-(257) × (3)-4-(6)μm. An elongate "branch," consisting of a second modified basidiospore with its germ tube arose from the septate region of the main tube (Fig. 5). Whereas tubes with two "branches" occasionally were observed in earlier studies (Gardner, 1981), only single-branched germ tubes were found in the present study. No major differences were found between teliospores germinated on water droplets and on water agar, although germ tubes produced on agar sometimes indicated a tendency to branch at their tips in later developmental stages.

Nuclear behavior Hyphal cells representing teliospore and aeciospore initials appeared binucleate, as were immature and mature aeciospores. Immature teliospores with binucleate pedicels were frequently uninucleate, indicating occurrence of karyogamy early in the development (Fig. 6). The single nucleus of the germinating teliospore migrated into the developing germ tube to a position midway to two-thirds of the tube length. A mitotic division, resulting in production of two daughter nuclei, occurred typically in the septate region as the germ tube lengthened. Less frequently, a subsequent mitotic division of one of the nuclei resulted in a total of three nuclei. Mitotic nuclei became delimited in individual cells by septation of the germ tube (Fig. 8). Initiation of septum formation was accompanied by increased fluorescence of cytoplasm in the septate region. The modified basidiospore developed as early as 4 h after initiation of germination at 20–24°C, into which the distalmost mitotic nucleus migrated (Fig. 9). The second nucleus migrated into the modified basidiospore of the developing "branch" (Fig. 10). Meiotic division occurred, or at least was initiated, in the modified basidiospore (Figs. 11, 12). Meiosis was characterized by production in rapid succession first of two, then four small closely associated nuclei, with a tri-nuclear intermediate stage also frequently visible. The meiotic nuclei migrated single file into the narrow germ tube (Figs. 13, 14). Completion of meiosis was evident as early as 16 h following initiation of germination at 20–24°C. Nuclear events in the main tube and in the "branch" occurred independently of each other, resulting in uncoordinated states of division within an individual germ tube.

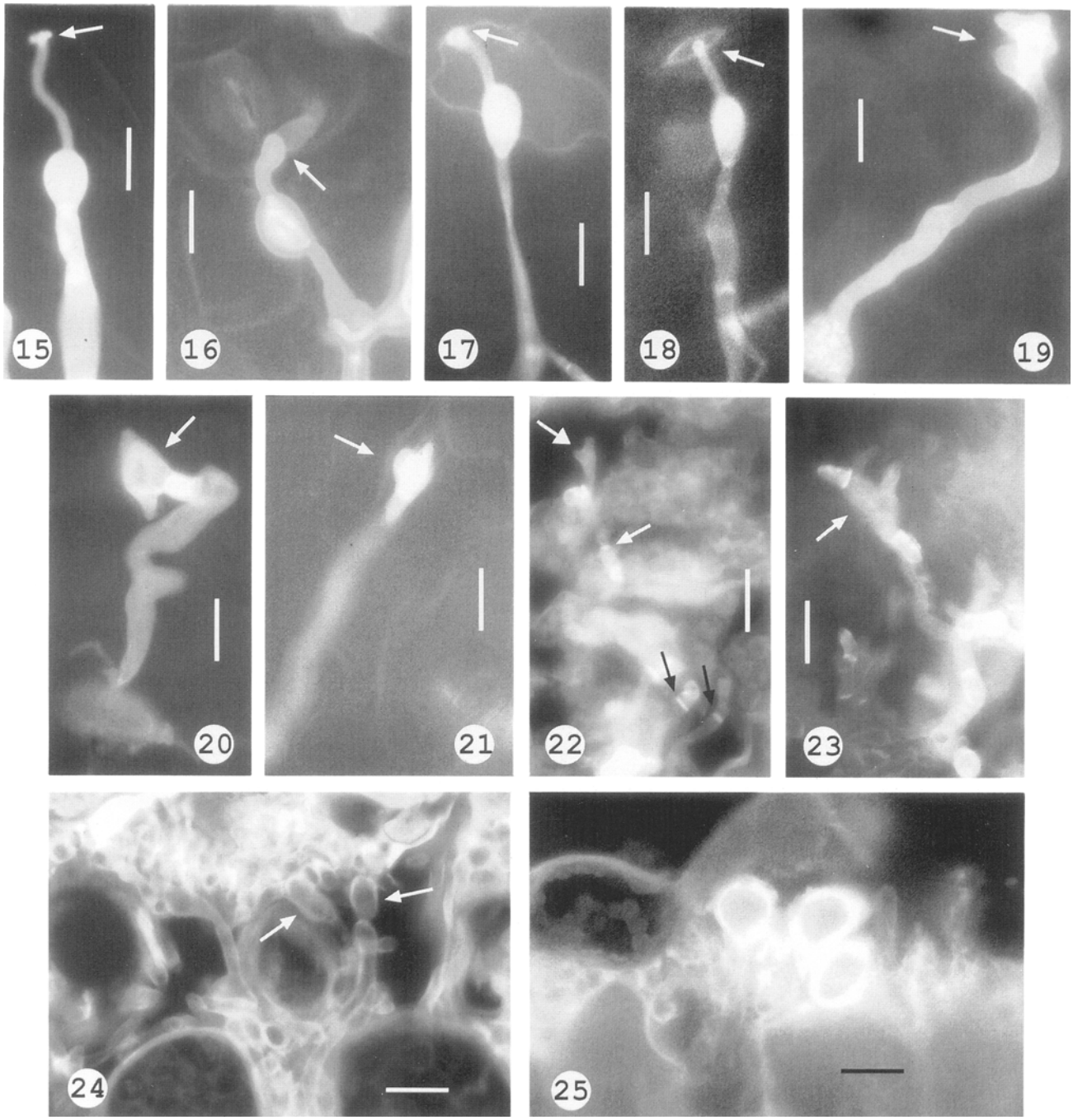
Table 2. Relation of leaf age to pustule number resulting from inoculation with *A. koae*.

Leaf age	Average pustules/100 Leaflets			
	Reps. (plants)			
	I	II	III	Mean
2 wk	11.9	5.6	4.8	7.4
8 wk	2.4	2.8	2.0	2.4
24 wk	0.6	0	0	0.2

Susceptibility of host tissue Success of infection was inversely correlated with leaf age. Approximately three times as many 2-wk old leaves as 8-wk old leaves became infected, and infection was nearly absent among 24-wk old leaves (Table 2).

Mode of penetration Spore germination on leaf surfaces and penetration were readily observed by staining with Calcofluor White. No morphological variations were noted among spores germinated on leaf surfaces as compared with those germinated on water droplets on microscope slides, but teliospores differed from aeciospores in their germination and penetration behavior. Hyphal tips of teliospores observed 48 h after onset of germination formed small triangular appressoria on the host tissue surface (Fig. 15). A small penetration peg was sometimes observed that pierced the cuticle and epidermal cell wall, or entered at the juncture of cell walls (Figs. 16, 17). Teliospore hyphae were also observed to enter host tissue through stomata (Fig. 18), but this mode of penetration was observed less frequently than was direct penetration.

In contrast to the direct penetration of teliospore



Figs. 15–25. *Atelocauda koae*. Host penetration and infection. 15. A small, triangular-shaped appressorium (arrow) of an infective hypha from a teliospore. 16. An infective hypha from a teliospore penetrating the host epidermis directly (arrow indicates point of entry) independently of a nearby stoma. 17. Direct penetration of an infective hypha from a teliospore at a cell junction (arrow). A reaction of the cell to penetration is indicated by Calcofluor White staining. 18. Less frequent entry of a teliospore infective hypha through a stoma (arrow). 19. A large, irregular-shaped appressorium (arrow) of an infective hypha from an aeciospore. 20. A large appressorium of an infective hypha from an aeciospore superimposed over a stoma (arrow) during entry of the penetration peg. 21. A penetration peg produced by a spindle-shaped aecial appressorium entering a stoma (arrow). 22. An intracellular hypha-like haustorium (large arrow). Haustoria delimited by prominent septa (small arrows) stained with Calcofluor White. 23. A large, digitate septate haustorium (arrow). 24. Masses of intercellular hyphae producing aeciospore and teliospore primordia (arrows). 25. Primordia developing into young aeciospores and teliospores breaking through the host epidermis. Scale lines = 20 μm in Figs. 15–25.

Fig. 26. *Atelocauda koae*. Sporulation on young *Acacia koa* plants resulting from the third in a series of successive inoculations with teliospores separated from aeciospores, demonstrating the ability of teliospores to perpetuate infection. Aeciospores produced no infection.

hyphae, germ tubes of aeciospores typically entered through stomata, in some cases accompanied by formation of appressoria and penetration pegs. The appressoria of aeciospores were triangular or spindle-shaped and somewhat larger than were those of teliospores (Figs. 19-21).

Internal fungal structures Masses of intercellular fungal material and coiled intracellular hyphae and haustoria were observed within teliospore-infected host tissue which had been prepared, sectioned, and stained with Calcofluor White as described above. Most haustoria were delimited by septa and were hypha-like (Fig. 22) or digitate (Fig. 23). In later developmental stages, i.e, 7-10 d after inoculation, conspicuous masses of fungal tissue were visible in substomatal spaces and formed aecial and telial primordia (Fig. 24), which eventually broke through the epidermis of the host to produce pedicels and spores (Fig. 25).

Infective ability Teliospores separated from aeciospores were capable of causing infection in healthy host tissue. Raised, chlorotic pustules became visible 7 d following inoculation, with sporulation beginning after 14 d at 23-25°C under continuous illumination at 5,000-7,000 lx. Resulting pustules contained spermogonia associated with a mixture of teliospores and aeciospores, with a predominance of teliospores, resembling the original sporulating material. Two additional consecutive inoculations with teliospores selected from these pustules resulted in similar infection (Fig. 26) producing telia, aecia, and spermogonia, demonstrating the repeating ability of teliospores. In contrast, whereas stomal penetration by aeciospore germ tubes was observed, inoculation with aeciospores did not result in detectable infection. Examination of cleared, Calcofluor White-stained tissue revealed no internal fungal material 30 d after inoculation with aeciospores.

Discussion

In the present study, no obvious dormancy requirement of *A. koae* teliospores was found. Whereas some of the variation in germination rates observed among field-collected samples (Table 1) may be functions of spore age, long-term dormancy did not appear to be a factor in the lower than 50% germination rate usually obtained. Gold and Mendgen (1983), in summarizing the effect of light on germination of rust teliospores, established three categories to include 1) spores which are not affected by light or darkness, 2) those which germinate in continuous dark but are inhibited by light, and 3) those which germinate in alternating treatments of light and dark. Teliospores of *A. koae* appeared to belong to those of the first category, but did not conform completely, since they germinated in both continuous light and darkness, although germination percentages were enhanced under light. The demonstrated preference of *A. koae* for young host tissue in the laboratory agrees with observations of fresh naturally-occurring infections in the field which are confined to young leaf, phyllode, or stem tissue.

Results of this study confirm Gardner's earlier observations (1981) of the unusual aspects of *A. koae*, including the lack of typical basidiospores. Later, in observing teliospore germ tubes of another endemic Hawaiian rust, *Puccinia vitata* Hennen & Hodges, which likewise did not produce typical deciduous basidiospores, Gardner (1988) recognized the vesicle-like swellings in the main germ tube and "branch" tubes of *A. koae* teliospores as probably representing vestiges of functional basidiospores. These structures were permanently attached, leaving the function of dispersibility to the teliospores themselves. The narrow tube extensions, representing germ tubes of the vestigial basidiospores into which the post-meiotic nuclei migrate, serve as infective hyphae capable of reinfecting the host. Hodges and Gardner (1984) suggested that the rusts of *Acacia* in the Pacific, now placed in the genus *Atelocauda*, were closely allied to one another and may have arisen from a common species, namely *A. digitata* from Australia. Presence of vestigial structures representing basidiospores supports the hypothesis that these rusts are in a transitional state toward reduced life cycles. The apparent occurrence of meiosis in these structures is noteworthy, since in other rusts meiosis typically occurs in the promycelium, followed by migration of one of the haploid nuclei into each of the four developing basidiospores. However, behavior somewhat reminiscent of that in *A. koae* recently was reported for two pine rusts, *Endocronartium sahoanum* Imazu & Kakishima and *E. yamabense* (Saho & I. Takahashi) Paclt (=*Peridermium yamabense* Saho & I. Takahashi), in which meiosis apparently resulted in production of a series of four nuclei in a vescile-like structure at the tip of the teliospore germ tube (Imazu et al., 1991). Recently, Gardner (1994) reported tetranucleate basidiospores, apparently resulting directly from meiosis, in another endemic Hawaiian rust, *Puccinia rutainsulara* Gardner. As earlier noted (Gardner, 1981), production of two or more diploid nuclei by mitosis, each of which is capable of in-

dependent meiotic division, appears to be unique to *A. koae*. This represents a significant addition to the eight variations in nuclear behavior of germinating rust teliospores illustrated by Hiratsuka and Sato (1982).

Whereas *A. koae* is now recognized as a demicyclic rust, producing spermogonial, aecial, and telial states, the simultaneous occurrence of aeciospores and teliospores suggests that this species does not undergo a normal life cycle in which these spore states are produced consecutively. The consistent mixture of teliospores with aeciospores has heretofore prevented assessment of the functional ability of each of these spore states in the life cycle. In this study teliospores were found capable of reinfecting the host and perpetuating the life cycle. On the other hand, although aeciospores were able to germinate and penetrate, their ability to establish infection appears to be greatly diminished or lost. This conclusion is supported by the observation of hypha-like or digitate haustoria which are thought to be produced by monokaryotic mycelia as compared with spherical or lobed haustoria which are associated with dikaryotic mycelia (Littlefield, 1981). Although the nuclear condition of inter- and intracellular hyphae was not observed, monokaryotic hyphae could be easily created by septation of the teliospore-produced infective hyphae, whereas hyphae arising from infection by aeciospores would be typically dikaryotic. *Atelocauda koae*, therefore, while morphologically appearing as demicyclic, may be considered functionally a microcyclic species. Microcyclic rusts are reduced forms consisting of a telial state, either accompanied or unaccompanied by spermogonia (Cummins and Hiratsuka, 1983; Petersen, 1974). As with other rusts of endemic Hawaiian hosts, including those of *Acacia*, *Atelocauda koae* is an example of unusual evolutionary diversity for which Hawaii, with its isolated insular environment, is well known.

Acknowledgements——We thank Robert Anderson for his assistance in collecting field material. This study was supported by funding from the National Biological Service, U.S. Department of the Interior.

Disclaimer——The use of trade names in this publication does not imply endorsement by the United States Department of the Interior to the exclusion of similar products which may be also acceptable.

Literature cited

Cummins, G. B. and Hiratsuka, Y. 1983. Illustrated genera of rust fungi. The American Phytopathological Society, St. Paul, Minn.

Gardner, D. E. 1981. Nuclear behavior and clarification of the spore stages of *Uromyces koae*. Can. J. Bot. **59**: 939–946.

Gardner, D. E. 1988. Teliospore germination of *Puccinia vitata*, an endemic Hawaiian rust. Mycologia **80**: 590–593.

Gardner, D. E. 1994. Teliospore germination and nuclear behavior of *Puccinia rutainsularа*, a microcyclic Hawaiian rust. Mycologia **86**: 486–493.

Gardner, D. E. and Hodges, C. S., Jr. 1985. Spore surface morphology of Hawaiian *Acacia* rust fungi. Mycologia **77**: 575–586.

Gold, R. E. and Mendgen, K. 1983. Activation of teliospore germination in *Uromyces appendiculatus* var. *appendiculatus*. Phytopath. Z. **108**: 267–280.

Hiratsuka, Y. and Sato, S. 1982. Morphology and taxonomy of rust fungi. In: The rust fungi, (ed. by Scott, K. J. and Chakrovorty, A. K.), pp. 1–36. Academic Press, New York.

Hodges, C. S., Jr. and Gardner, D. E. 1984. Hawaiian forest fungi. IV. Rusts on endemic *Acacia* species. Mycologia **76**: 332–349.

Imazu, M., Kakishima, M. and Katsuya, K. 1991. Morphology, cytology, and taxonomy of stem rusts on five-needle pines in Japan. In: Rusts of pine, (ed. by Hiratsuka, Y., Samoil, J. K., Blenis, V., Crane, P. E. and Laishley, B. L.), pp. 76–91. Proc. 3rd IUFRO Rusts of Pine Working Party Conference, Sept. 18–22, 1989, Banff, Alberta, Canada. Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta. Information Report NOR-X-317.

Jensen, W. A. 1962. Botanical histochemistry: Principles and practice, W. H. Freeman and Co., San Francisco.

Kang, Z., Shang, H. and Li, Z. 1993. Fluorescent staining technique of wheat rust tissue. Plant Protection 19: 27.

Littlefield, L. 1981. Biology of the plant rusts: An introduction. Iowa State University Press, Ames.

Petersen, R. H. 1974. The rust fungus life cycle. Botan. Rev. **40**: 453–513.

Stevens, F. L. 1925. Hawaiian fungi, Bernice P. Bishop Museum Bulletin No. 19. Honolulu, Hawaii.

Whitesell, C. D. 1990. *Acacia koa* A. Gray. Koa. In: Silvics of North America, vol. 2, Hardwoods, (ed. by Russell, M. B. and Honkala, B. H.), pp. 17–28. Washington, D.C.