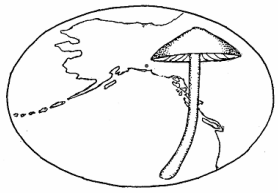
# Pacific Northwest Fungi



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# Mycoflora of seed of common teasel (*Dipsacus fullonum*) in Washington State

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**Abstract:** Seeds of standing common teasel (*Dipsacus fullonum*) were harvested in January 2007 in Pullman, Washington, and divided into two categories, symptomatic versus asymptomatic, on the basis of signs of fungal colonization at 10-50X magnification. The most common signs were pseudothecia of *Davidiella tassiana*. Fungi were recovered from all seeds of both categories when seeds were surface-disinfested and incubated on agar media. *Aureobasidium pullulans* accounted for 57-72% of fungal isolates from asymptomatic seed, but only 16-26% of isolates from symptomatic seed. *Cladosporium* spp. and *Alternaria* spp. exhibited a combined frequency of 54-64% from symptomatic seed, versus 16-35% from asymptomatic seed. Asymptomatic seed germinated at incidences of 44-76% whereas symptomatic seed germinated at incidences of 2-6%. When seeds or germinated seedlings were inoculated with conidial suspensions of representative isolates of *Au. pullulans*, *C. herbarum* (anamorph of *D. tassiana*) or *Alternaria* sp., and incubated under conditions favorable for germination or growth of teasel, no differences were apparent between treated seed and non-inoculated control seed. The correlation between colonization by *Alternaria* and *Cladosporium* species and diminished germination ability probably reflects unidentified, predisposing factors for diminished germination. Immature pseudothecia of *D. tassiana* were repeatedly observed to germinate directly on the seed by production of fertile conidiophores from the apices of the papillae.

**Key words:** *Alternaria*, *Aureobasidium pullulans*, biological control, *Cladosporium herbarum*, *Davidiella tassiana*, *Dipsacus fullonum*, *D. sylvestris*, *Mycosphaerella*, teasel, weed.

**Introduction:** Common teasel (*Dipsacus fullonum* L, syn. = *D. sylvestris* Huds.) is a well established, invasive weed throughout North America, present in 43 states and listed as a noxious weed in Colorado, Iowa, Missouri and New Mexico (USDA National Resources Conservation Service n.d.). It is of European origin and was likely introduced to North America on multiple occasions over the course of two centuries as a seed contaminant of cultivated teasel [*D. sativus* (L.) Honckeny], a minor crop that is now obsolete (Rector et al, 2006). The Pacific Northwest of the United States was once the primary American region for commercial production of cultivated teasel (Courtney 1952), and common teasel is widely distributed in this region. Because the plant is biennial, i.e., it does not set seed until the second year, *D. fullonum* can be controlled by repeated mowing or tillage, but can be persistent and even dominant in areas where such control measures are impractical. *Dipsacus fullonum* is also the classical host of the cosmopolitan nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, a plant pathogen with a wide host range, including alfalfa, garlic, onion and other commercial crops, and sometimes the object of plant quarantines (e.g., EPPO n.d.; Nevada Department of Agriculture n.d.).

A list of natural enemies potentially effective as biological control agents against common teasel has been published (Rector et al. 2006). Effective and environmentally sound biological control would be more probable with such agents that are both highly aggressive and host-specific. Successful use of biological control agents could be aided by improved knowledge of the biology and microbial ecology of *D. fullonum* in the invaded range. Assuming the relevance of the latter, and given the importance of seeds in the life cycle, we embarked on a preliminary survey of seed mycoflora of common teasel in the Pacific Northwest. Specifically, we documented differences in mycoflora between seed that appeared healthy and seed that bore signs of colonization by fungi, and tested for correlation of such signs or symptoms with reduced incidence of seed germination.

During the course of this study we also noticed what appears to be a previously unrecorded method of germination of sexually immature *Davidiella tassiana* (De Not.) Crous & U. Braun (= *Mycosphaerella tassiana* (De Not.) Johanson) via the production of fertile conidiophores directly from apical cells of the pseudothecial papillae. Since *D. tassiana* was among the most common colonizers of teasel seed, we include these observations in this report.

**Materials and methods:** *Visible colonization of seed*: On 18 and 19 Jan 2007, ca. 6 liters of teasel inflorescences (1-4 inflorescences removed per plant) were harvested at each of two locations (TD = intersection of Grand Avenue and NW Turner Drive, and DQ = intersection of Grand Avenue and Bishop Boulevard, respectively) in Pullman, Washington. Approximately 20-25 plants comprised each sample. Gentle agitation readily extracted ca. 30 g dry seed from each collection. Seed from each location was examined at 10-50X magnification and sorted into two categories: asymptomatic and symptomatic, with criteria for the latter being conspicuous colonization of the seed by fungi, e.g., the presence of immature pseudothecia (fruiting bodies). Approximate assessments were also made of visible seed colonization at each location by dividing a random sample of slightly >100 seeds into classes of: i) asymptomatic, ii) 1-4 fruiting bodies evident per seed, iii) >4 fruiting bodies per seed, and iv) >4 fruiting bodies per seed plus any other signs of seed deterioration. The category of symptomatic above corresponded roughly to combined classes iii and iv. Additional collections were made at DQ on 24 March and 24 April, and at a third

location (PO = intersection of Grand Avenue and Crestview St.) on 12 May in order to monitor maturity of the pseudothecia that accounted for the majority of signs on the seed. All sampling locations were situated on slight, south-facing inclines approximately 20 yards from a major roadway (Grand Ave.) and 20 to 75 yards from small creeks with riparian vegetation. The DQ location was adjacent to several acres of cultivated wheat on one side, whereas the TD and PO locations were in weedy areas dominated by teasel and totally surrounded by roads, buildings and other urbanized structures.

*Assay of seed for fungi*: Seeds of each category from each location were dipped in 70% ethanol for 30 seconds and rinsed in sterile de-ionized water. Five seeds were placed onto each of 10 culture dishes of half-strength V8 agar (½V8, Stevens 1981) amended after cooling with 50 µg/ml streptomycin sulfate and 50 µg/ml tetracycline hydrochloride, for a total of 50 seeds from each category and location. The seeds were incubated under periodic fluorescent light (12 hr/12 hr) at ambient laboratory temperature (ca. 22° C) until fungal colonies were observed. Characters of colonies (color, texture, margin, growth rate) were recorded. The numbers and relative sizes of each type of colony were recorded, and transfers were made from a sample of representative colonies to fresh slants of ½V8 for preservation and storage at -80°C. Results were analyzed statistically on the basis of most prevalent colony type from each seed, as well as on the basis of all recorded colony types (often >1 per seed).

*Identification of isolates*: Representative isolates of the most commonly recovered taxa were identified to genus or species by standard morphological and cultural criteria (Andersen and Thrane 1995; de Hoog and Yurlova 1994; Ellis 1971, 1976; Hermanides-Nijhof 1977; Ho et al. 1999; Simmons and Roberts 1993).

*Correlation of colonization with germination ability*: To document the degree to which visible colonization of the seed by fungi might diminish the incidence of seed germination, 100 seed of each category (symptomatic, asymptomatic) from each location (DQ, TD) were subjected to the surface-disinfestation protocol described above, and placed into germination chambers. Each chamber consisted of a glass Petri dish containing three layers of moist filter paper onto which were placed 10 seeds, and the plates were sealed with Parafilm® (Pechiney Plastic Packaging, Chicago, Illinois). The seeds were incubated as described above, and the number of seeds germinated in each chamber was recorded after two weeks.

*Identity and sexual maturity of pseudothecia, and production of conidiophores directly from the pseudothecial apex*: Pseudothecia were excised from seed, and occasionally from floral bracts, from collections at all dates and locations, and examined at 100-400X magnification for diagnostic characters. Five immature pseudothecia bearing conidiophores at the apex, and six without conidiophores, were surface-disinfested by dipping each pseudothecium for 2-3 seconds in 70% ethanol followed by dipping in sterile distilled water. The pseudothecia were then transferred to malt extract agar amended with antibiotics as described above, and incubated as described above.

*Examination of material from BPI*: Specimens of *Mycosphaerella* collected on *Dipsacus* in Pullman by G.G. Hedgcock in 1934 and 1935, and deposited as *M. dipsaci* Dearn. BPI 608487 and BPI 608489, respectively, were examined microscopically for possible relationship to *D. tassiana*.

*Statistics*: Results were analyzed by analysis of variance with Systat 9.0 (SPSS Inc., Chicago, Illinois), with relative probabilities reported as LSD.

**Results:** *Visible colonization of seed*: At the DQ location, 24% of the seed bore no external signs of colonization by fungi, 43% of the seed had 1-4 fungal fruiting bodies, 19% had >4 fruiting bodies, and 14% had >4 fruiting bodies plus other signs of seed deterioration. At the TD location, the corresponding results were 29, 36, 17 and 18%, respectively. The most conspicuous sign of fungal colonization was the production of papillate pseudothecia. When crushed and examined at 100-400X magnification, none contained spores. Later harvest of material (24 March) for the purpose of following development of these bodies gave the same result. However, some of the March samples, as well as samples harvested in January and incubated at ca. 25°C with high humidity, produced clusters of conidiophores with conidia at the very apex of the pseudothecia (Figs. 1,2). The conidiophores originated from apical cells of the papilla (Figs. 3,4). The conidiophores and conidia were morphologically consistent with *Cladosporium herbarum* (Pers.: Fr.) Link. Conidiophores and conidia of *C. herbarum* not directly associated with pseudothecia were also common on incubated seed. A *Camarosporium* sp., never recovered into culture during seed assays on artifical media (below), was sometimes detected on seed from the DQ location.

*Assay of seed for fungi*: The most common taxa recovered in culture were *Alternaria* isolates of the *A. alternata - A. tenuissima* complex, *Aureobasidium pullulans* (de Bary) G. Arnauld, *Cladsporium* cf. *cladosporioides*, *C. herbarum*, *Epicoccum nigrum* Link, and *Ulocladium* spp. Non-sporulating fungi and an unidentified coelomycete were present at much lower frequencies. *Cladosporium herbarum* was observed more frequently than other *Cladosporium* species. The growth of fungal colonies from seed samples at each of the two locations and for the two categories of seed revealed the prevalence of *Au. pullulans* over *Alternaria* and *Cladosporium* spp. in asymptomatic seed. This was true with regard to the most prevalent colony type from each of 50 seeds (Table 1), and for the total number of fungal colonies growing on each seed, i.e., counting all colonies including instances in which a given seed produced >1 type of colony (Table 2).

**Table 1.** Primary fungal taxa isolated from common teasel seed from Washington State on the basis of most prevalent colony observed on each of 50 seeds (5 seeds/plate x 10 plates) on 1/2 V8 agar medium. For each genus, differences with regard to condition (symptomatic vs. asymptomatic) were significant and are specified in the text; differences between locations were not significant.

| **DQ asymptomatic** | | **TD asymptomatic** | |
|---|---|---|---|
| *Aureobasidium* | 36 | *Aureobasidium* | 32 |
| *Cladosporium* | 0 | *Cladosporium* | 6 |
| *Alternaria* | 8 | *Alternaria* | 9 |
| Other | 6 | Other | 3 |
| Total | 50 | Total | 50 |
| **DQ symptomatic** | | **TD symptomatic** | |
| *Aureobasidium* | 13 | *Aureobasidium* | 10 |
| *Cladosporium* | 14 | *Cladosporium* | 13 |
| *Alternaria* | 13 | *Alternaria* | 16 |
| Other | 10 | Other | 11 |
| Total | 50 | Total | 50 |

With regard to the most prevalent fungal colonies observed on the seed, the mean number of *Au. pullulans* colonies from 5 seeds was 3.40 for asymptomatic seed, versus 1.15 for symptomatic seed (P = 0.000); there were no significant differences between locations. The mean number of colonies of *Cladosporium* spp. from 5 seeds was 0.30 from asymptomatic seed, which differed from a mean of 1.35 from symptomatic seed (P = 0.001); there were no significant differences between locations. Corresponding means for *Alternaria* isolates were 0.85 colonies from 5 asymptomatic seeds, and 1.45 colonies from 5 symptomatic seeds (P = 0.077); with no significant differences between locations. The colony types lumped under "other" were also less frequent on asymptomatic

**Table 2.** Primary fungal taxa isolated from common teasel seed on the basis of total number of colonies from each of 50 seeds (5 seeds/plate x 10 plates) on 1/2 V8 agar medium. For each genus, differences with regard to condition (symptomatic vs. asymptomatic) were significant and are specified in the text; differences between locations were not significant.

| **DQ asymptomatic** | | **TD asymptomatic** | |
|---|---|---|---|
| *Aureobasidium* | 40 | *Aureobasidium* | 41 |
| *Cladosporium* | 5 | *Cladosporium* | 14 |
| *Alternaria* | 9 | *Alternaria* | 11 |
| Other | 7 | Other | 6 |
| Total | 61 | Total | 72 |
| **DQ symptomatic** | | **TD symptomatic** | |
| *Aureobasidium* | 17 | *Aureobasidium* | 12 |
| *Cladosporium* | 36 | *Cladosporium* | 29 |
| *Alternaria* | 20 | *Alternaria* | 16 |
| Other | 14 | Other | 16 |
| Total | 87 | Total | 73 |

seed (mean 0.45 colonies per 5 seeds) than on symptomatic seeds (mean of 1.05) (P = 0.030), with no significant effect of location.

With regard to the total number of fungal colonies observed on the seed, the mean number of *Au. pullulans* colonies growing from 5 seeds was 4.05 for asymptomatic seed versus 1.45 for symptomatic seed (P = 0.000); but total counts between locations were not significantly different. The mean number of colonies of *Cladosporium* spp. growing from 5 seeds was 0.95 from asymptomatic seed, which differed significantly from a mean of 3.25 for symptomatic seed (P = 0.000); differences between locations were not significant. Corresponding means for *Alternaria* isolates were 1.00 colonies from 5 asymptomatic seeds, and 1.8 colonies from 5 symptomatic seeds (P = 0.05); location effects were not significant. The colony types lumped under "other" were less frequent on asymptomatic seed (mean of 0.65 colonies per 5 seeds) than on symptomatic seeds (mean of 1.50) (P = 0.01); location effects were not significant. Fungi included in the category "other" were primarily *Epicoccum* and *Ulocladium* spp. plus some unidentified fungi. Thus, colonization by *Au. pullulans* was positively correlated with an asymptomatic seed condition, but colonization by *Alternaria* or *Cladosporium* spp. was correlated with a symptomatic seed condition, regardless of location.

*Association of colonization with germination ability*: The incidence of seed germination differed significantly by location (DQ > TD, P = 0.022) and based on condition of the seed (symptomatic vs. asymptomatic, P = 0.000) (Table 3).

*Identity and sexual maturity of pseudothecia, and production of conidiophores directly from pseudothecial apices*: No asci or ascopores were observed in pseudothecia found on common teasel seeds until the PO collection of 12 May. Pseudothecial habit, size, wall and centrum characters, asci (Fig. 5) and ascospores (Fig. 6) were congruent with *D*. (*Mycosphaerella*) *tassiana* as described and illustrated in detail by Corlett (1988). Pseudothecia bearing typical *C. herbarum* conidiophores and conidia at the apices of the papillae, when surface-disinfested

**Table 3.** Germination incidences of common teasel seed by location (DQ and TD near Pullman, WA) and the presence (symptomatic) or absence (asymptomatic) of fungal colonization on the seed. Differences between condition (asymptomatic vs. symptomatic) and location were significant and are specified in the text.

| | |
|---|---|
| DQ asymptomatic | 76% |
| DQ symptomatic | 6% |
| TD asymptomatic | 44% |
| TD symptomatic | 2% |

and transferred to agar, produced typical *C. herbarum* colonies for three of five pseudothecia subjected to this process. For one of the pseudothecia, the resulting colony was

*Cladosporium*-like but non-sporulating. For another pseudothecium, which was subjected to a slightly longer disinfestation time, no fungal growth was observed. One of the three colonies of *C. herbarum* was accompanied by some growth of *Au. pullulans*. Immature pseudothecia lacking such manifestations of asexual reproduction, when disinfested and transferred in the same manner, produced colonies typical of *C. herbarum* for five of six pseudothecia; the sixth immature pseudothecium was subjected to a slightly longer disinfestation time and developed no fungal growth in culture. For four of the five pseudothecia from which *C. herbarum* was produced, the cultures also contained *Au. pullulans.*

*Examination of material from BPI*: Dimensions, habit and microscopic characters of pseudothecia, pseudothecial walls, centrum, asci and ascospores of Hedgcock's material were congruent with *D. tassiana* as described in Corlett (1988).

**Discussion:** *D. fullonum* is a prolific producer of seed, which exhibits rates of germination of ca. 90% in mid-autumn after the seed is produced. However, in nature "few seedlings are found until the germination pulse the following spring" (Werner 1975a). There are indications that successful establishment of seedlings from germinating seed of common teasel may be impaired by damping-off induced by fungi. Werner (1975b) noted marked variations in survival of seedlings germinated under various types of litter, with rotting of tissues most highly correlated by exposure to forb litter. *Alternaria* spp., and to a lesser extent *Cladosporium* spp., are agents of seed infection and/or damping-off of various plants (e.g., Agarwal and Sinclair 1997; Hutchins and Reeves 1997; Richardson 1990; Rotem 1994; Singh and Mathur 2004). Although *Au. pullulans* can induce plant disease, this usually happens in ripe fruits (e.g., Dugan and Roberts 1994), and elsewhere this common phylloplane fungus is regarded as typically saprobic (Domsch et al. 1993; Hermanides-Nijhof 1977). *Au. pullulans* has also been repeatedly exploited for experimental biological control of fungal plant pathogens (e.g., Dugan et al. 2005; Schena et al. 1999). Of the fungi reported as natural enemies by Rector et al. (2006), none were identified in this study, although Dugan and Glawe (2006) reported the powdery mildew, *Sphaerotheca dipsacearum*, from the Pullman locale.

The results of this study indicated a high rate of colonization of symptomatic seed of common teasel from Washington State by *Cladosporium* and *Alternaria* species, versus a high rate of colonization of asymptomatic seed by *Au. pullulans.* These results conform to the reputation of these species in the literature, as indicated above. However, when suspensions of conidia ($10^5$/ml) of representative isolates of *Au. pullulans*, *Alternaria* sp., or *C. herbarum* were applied to seeds or germinated seedlings incubated under conditions favorable for germination (on damp but not flooded filter paper, and ca. 22-25°C under periodic artificial light), no significant differences were apparent between inoculated seed or seedlings and non-inoculated control seed or seedlings (replicated trials of 30+ seeds/rep for each treatment; data not shown). Such results are not surprising for *Au. pullulans*, but some damping-off was expected for the *C. herbarum* and *Alternaria* isolates. The extensive literature on damping-off of various plants by *Alternaria* and *Cladosporium* spp. is probably correct, as were the observations of Werner (1975b), but conditions more adverse to the host than provided in this study may be necessary for damping-off to occur in teasel seed or seedlings. The isolates used in this study were apparently not aggressive pathogens. We have no explanation why asymptomatic seed from the TD location germinated less frequently than comparable seed from the DQ location (Table 3), since the two locations were approximately a kilometer apart and of essentially identical

elevation and aspect. Some minor differences between sites are described in Methods.

The representative isolates of *C. herbarum* and *Alternaria* sp. tested for pathogenicity on teasel proved non-aggressive and failed to induce any damping-off. But the association of seed colonization by these taxa with failure of visibly colonized seeds to germinate was, statistically, extremely robust (Tables 1-3). Moreover, the most visible sign of colonization, which was highly correlated with inability of the seed to germinate, was the presence of *D. tassiana*, teleomorph of *C. herbarum*. We do not know which underlying factors are responsible for these associations. Possible explanations are that a combination of environmental and phenological factors rendered the seed susceptible to infection early in development on the mother plant, at which time the *Cladosporium* and *Alternaria* species were able to infect the seed. Perhaps prolonged wetting during the drying phase of maturation could have rendered seeds susceptible to colonization at that time, with resultant subsequent deterioration. Or, perhaps an alternative pathogenic agent (possibly obligate, and therefore not detected in our seed assays) or pest compromised defenses of the seed and rendered the seed susceptible to colonization by these fungi.

The pseudothecia, which were the most conspicuous sign of fungal colonization of the seed, were not observed to produce asci or ascospores until seed samples were collected on 12 May, but at that time were convincingly identified as *D. tassiana*, teleomorph of *C. herbarum*. *Cladosporium herbarum* has been described from old carpophores of various agarics, a *Mitrophora* species, and several *Exobasidium* species (the latter records probably reflective of *Cladosporium exobasidii* Jaap, a morphologically distinct species), but is considered a saprobe (Heuchert et al. 2005). The conidiophores of *C. herbarum* associated with pseudothecia on common teasel seed originated directly from the apical cells of the papillae of the pseudothecia (Figs. 3,4).

The subsequent identification of the mature pseudothecia as *D. tassiana*, the remote possibility of mycoparasitism by *C. herbarum*, and the apparent physical origin of the conidiophores from papillae cells, renders nearly inevitable the conclusion that immature pseudothecia were "germinating" by production of conidiophores. Nonetheless, additional support for this hypothesis was gained by confirming that *C. herbarum* was present in all fungal growth from surface-disinfested, immature pseudothecia transferred to an agar medium. It was difficult to surface-disinfest individual pseudothecia effectively and still retain viability (hence the concomitant survival of *Au. pullulans* on some surface-disinfested pseudothecia and the lack of fungal growth from others). However, *C. herbarum* was produced from every disinfested, immature pseudothecium from which sporulating colonies were recovered. This, together with the above evidence, confirms that production of *C. herbarum* conidiophores and conidia from the pseudothecia represents a form of germination, and not incidences of mycoparasitism by *C. herbarum*. The production of conidiophores and conidia from *D. tassiana* represents the same association that has been observed with other ascomycetes, wherein the anamorph can originate on the ascomata or associated stromata. Analogous "germination" can occur in *Pleospora herbarum* (Pers.:Fr.) Rabenh., whereon conidiophores and conidia of *Stemphylium* are sometimes produced near the pseudothecium apex (L. du Toit, personal communication).

No records are associated with the name *M. dipsaci* in the Index Fungorum (www.indexfungorum.org), nor are records other than the BPI specimens associated with that name in the databases of the USDA- ARS

Systematic Botany and Mycology Laboratory (nt.ars-grin.gov/fungaldatabases/index.cfm). The name '*Mycosphaerella dipsaci* Dearn.' appears on the labels for both specimens collected on *Dipsacus* in Pullman by G.G. Hedgcock in 1934 and 1935. Dearness is listed as the determiner for BPI 608489, but it seems that Dearness never published the name.

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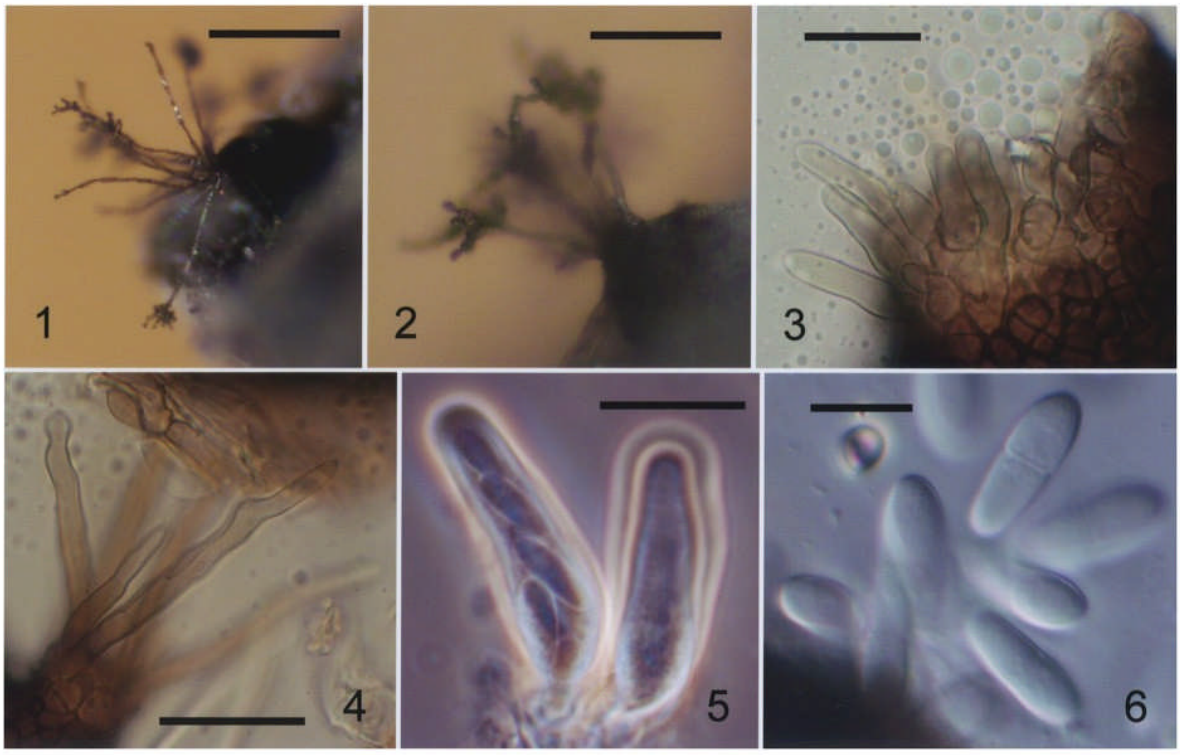


Fig. 1. Conidiophores and conidia typical of *Cladosporium herbarum* originate from the apices of otherwise sterile or immature pseudothecium-like bodies growing on common teasel seed. Bright field. Scale bar = 150 µm. Fig. 2. Illustration of the same structures as Fig. 1, but with pseudothecium partially immersed in a bract. Bright field. Scale bar = 130 µm. Fig. 3. Conidiophores appear to originate from apical cells of the papilla of a pseudothecium-like body. Bright field. Scale bar = 20 µm. Fig. 4. Immature conidiophores at the apex of a pseudothecium-like body. Bright field. Scale bar = 35 µm. Fig. 5. Asci of *Davidiella tassiana*. Phase contrast. Scale bar = 25 µm. Fig. 6. Ascospores of *Davidiella tassiana*. Differential interference contrast. Scale bar = 10 µm.