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Archaeobotanical evidence of the fungus Covered smut (*Ustilago hordei*) in Jordan and Egypt

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Archaeological evidence for the infection of barley with the fungus Covered smut (Ustilago hordei) was first discovered in the gut contents of two Danish bog bodies dated to the Iron Age. The fungus was recognized by its spores. New evidence, in the form of infected rachis fragments, originates from the Roman sites of Berenike and Shenshef in Egypt, excavated by Leiden University (the Netherlands) and the University of Delaware (USA). Subsequent records of this plant disease relate to additional Roman and post-Roman sites in Egypt: Roman Karanis and the Roman and Islamic ports at Quseir Al-Qadim. However, the recent discovery of Ustilago hordei infection in samples containing charred barley from tell Dayr Alla in Jordan, also excavated by Leiden University, shows that the fungus was already present in the Southern Levant during the Iron Age.

The percentage of infected rachis within samples from Berenike, based on counts of the rachis nodes, varies from 0.3% to 11.7%. All records of infected barley concern the hulled 6-row subspecies (Hordeum vulgare ssp. vulgare).

1 Introduction

Archaeobotanical evidence for barley is based on the recovery of grain kernels and threshing remains. The composition of the threshing remains depends on the husk tightness. In hulled barley, threshing results in grain kernels still enclosed by their chaff (lemma and palea); only the fragile awns and glumes are broken off and become part of the threshing remains. In free-threshing (or naked) barley, the palea and lemma are also separated from the grain and end up in the threshing remains. With both hulled and free-threshing barley, fragments of the culm and the rachis also become part of the threshing remains; the proportion of culm fragments is related to the reaping height.

Rachis fragments are of special interest because they can be used for identifying the subspecies and for quantifying the proportion of recovered cereals. In contrast to the culm, from which only the nodes are solid, in the rachis, both nodes and internodes have a dense structure. Rachis fragments can, therefore, be well preserved even in charred condition. The presence of pedicels on the edges of the nodes can be used to distinguish 2-row Barley (ssp. *distichon*) from 6-row Barley (ssp. *vulgare*). In 2-row Barley, both lateral spikelets are pedicellate (stalked), whereas in 6-row Barley, the lateral spikelets are mostly sessile (for a discussion, see Cappers and Neef 2012, 276). Although a subdivision of barley beyond the species level is not always accepted (*e.g.*, Van der Meijden 2005), in our opinion, identifying barley to the level of subspecies makes sense because it can be indicative of its use and because it can aid in quantifying the proportion of cereals (Cappers *et al*. 2004; Cappers and Neef 2012, 410).

It has only recently been recognized that rachis fragments can also be used to identify former crop diseases. This article deals with the infection of barley with the fungus Covered smut (*Ustilago hordei* [Pers.] Lagerh.). The infection leaves marks on the rachis fragments that are so prominent that the shape of the rachis becomes obscure, and sometimes the rachis is completely covered with the fungus. The morphological transformation of the rachis is most probably the reason why this plant disease is not always recognized. Here, we present the first archaeobotanical evidence for Covered smut from sites that have been excavated by Leiden University, namely, Dayr Alla in Jordan and Berenike and Shenshef in Egypt.

2 Archaeobotanical evidence of Covered smut infection

Crop yields of cereals can be reduced by several plant diseases, including smut fungi. Barley is affected by three different smut fungi, namely, Loose smut (*Ustilago nuda* [Jens.] Kellerm. et Swingle), Black (or False) loose smut (*U. nigra* Tapke) and Covered smut (*U. hordei*). Covered smut produces sori that are covered by a membrane until maturity. Loose smut produces a thin membrane that easily disintegrates when the infected spikes develop, thus releasing the spores. The specific smut fungus present on infected subfossil rachis fragments can be easily identified by examining the surface of the spores. Spores of Covered smut have a smooth surface, whereas those of Loose smut are finely reticulate. J.P. Meffort, of the Plant Protection Service, Ministry of Economic Affairs, Agriculture and Innovation, Wageningen, the Netherlands, checked the spores present on the subfossil rachis fragments discussed here and confirmed the identification of Covered smut.

According to Briggs (1978, 359), infection with Covered smut occurs in the field, when the membranes split and spores are dispersed by the wind to neighbouring spikes, and on the threshing floor, where threshing tools are used to fragment the spikes into individual spikelets. Because the membranes do not break up readily, most of the infection happens during threshing. When the spore masses are released on the threshing floor, they can easily infect healthy grain kernels; the fungus is thus effectively dispersed through seeds used for sowing. Spores present in the topsoil and on the grain kernels germinate and infect the seedlings of barley at an early stage. The fungus grows in the barley plant and produces new spores in the spike, where all or most of the grain kernel and chaff is replaced by fungal spores.

Subfossil spores from covered smut were discovered by Helbaek in the gut contents of two Danish bog bodies, known as Tollund man and Grauballe man, both dated to the Iron Age (Van der Sanden, 1996). Although the spores are indicative of the presence of the fungal infection, they do not allow for an estimate of the infection rate. This is only possible by comparing the proportion of infected rachis nodes to non-infected rachis nodes.

3 Evidence from written sources

Following Theophrastus' (4th–3rd c. BC) classification, phytopathology was regarded as a deviation from the well-being (εύθένεια) of a plant, that is, a certain fit between plant ecological requirements, the environment and the adopted agricultural practices. References to plant diseases are frequent in classical written sources that describe distinct characteristics of plants and agricultural practices. These include works that provide first-hand botanical observations and evidence of crop husbandry, as well as compilations of previous writings under a different authorship. The first group includes the botanical treatises by Theophrastus and the agricultural treatises by Cato (3rd–2nd c. BC), Varro (2nd–1st c. BC) and Columella (1st c. AD); the second group is well represented by the encyclopaedic work of Plinius the Elder (1st c. AD), which often shows a clear preference for spells and hearsay.

Fungal diseases such as rusts and smuts in particular captured the attention of ancient writers. However, the interpretation of these early records is complicated by their authors' use of general terms, often with overlapping meanings, which means that sometimes little can be deduced from the context. The Greek word ἐρυσίβη (erysibê) is used to define a plant fungal disease that is described as a kind of decomposition due to water, such as rainwater and dew, being retained on the plant surface, and which is promoted by heating (Theophrastus, *C.P.,* IV. 14.3). Related to this belief is the idea of an "aerial or atmospheric origin" of the disease (νόσος τις <ύξ> ύέρος, Hesychius, *Lexicon* (A-O), E. 6111.1; cf. "caeleste malum", Plinius, *N.H.*, XVIII, 154). With reference to cereals, ἐρυσίβη is generically translated as rust (viz. Uredinales) or smut (viz. Ustilaginales). Generic terms are sometimes related to diseases of particular plants, such as rot (σφακελισμύς, Theophrastus, *H.P.* VIII. 10.1) for chick-pea and mildew (ύλμύ, literally "become mildewed", *Ibid.;* cf. Theophrastus, *C.P.* VI.10.5) for cumin. In these cases, a connection might be suggested with highly noxious fungal diseases such as dry root rot in chick-pea and wilt and blight in cumin (for coriander, cf. Theophrastus, *H.P.* VII. 5.4). In other cases, the use of general definitions such as "canker" (ύνια δύκαύ ψωριύ, Theophrastus, *H.P.* VIII. 10.1) does not even allow us to ascertain the fungal origin of the disease under discussion.

In Latin, the word *rubigo* (or *robigo*) is in general translated as rust (*Puccinia graminis*?), which is regarded as a major disease of cereals ("frugum rubigo, quidem maxima segetum pestis", Plinius, *N.H.*, XVIII, 161), while the words *uredo* and *carbunculus* can be related, respectively, to blight or smut on grasses and to coal-blight, especially in vines (*carbunculus vitis*). Of particular interest is a description provided by Plinius (*N.H.*, XIX, 181 s.) of a poorly defined barley disease that arises when grains that are not yet ripe or are at the first stage of ripening become hollow and empty and fade away in the ear.

While emphasizing the uncertainty surrounding the results of agriculture practices, Xenophon (*Oeconomicus*, V 18.5) mentions ἐρυσἐβαι among the major causes of crop loss. Protection against ἐρυσίβη was sought through worshipping divinities considered to be protectors of cereal crops. Strabo (*Geogr.*, VIII 3.15) refers to the great importance attached to the worship of Demeter and Core, the cereal harvest goddesses, in the Greek region of Messenia, where often there was no crop yield at all because of the disease. On the island of Rhodos, Apollos *Erythibius* (literally, "averting rust/smut") was propitiated as preventer of the pest (Strabo, XIII 1.64). Among the Romans, the pest itself was worshiped as Robigo/Rubigo.

Theophrastus (*H.P.* VIII, 10.2) mentions different levels of liability of plants to ἐρυσίβη and summarizes the reasons. Cereals are more susceptible than pulses, and among cereals, barley (κριθύ) more than wheat (πυρός). Among barleys, some kinds are more susceptible than others to the infection. This is the case for a variety called "Achillean" (ύχιλληΐς χριθή), the relatively erected ears of which would have caused rain and dew to remain on the plant longer because the ears stand off from the leaves. In contrast, the barley variety called ἐτεόκριθος (literally, "genuine, good barley") is mentioned as being less susceptible to the disease because its ears are more bent and thus allow the water to be removed, especially in elevated (ἔγκοιλα) and windy locations (Theophrastus, *C.P.* 3.22.2; cf. Theophrastus, *H.P.* VIII 4.2). The "Achillean" is defined as a plump and large variety of hulled barley (Hippocrates, *Diseases III,* 17

(Loeb vol. VI, 59) able to withstand dry conditions and sandy soils (Theophrastus, *C.P.* III.21.3). A possible indication about its origin is given in Galen's *Hippocratic Glossary* (*Linguarum seu Dictionum Exoletarum Hippocratis Explicatio*) (Vol. XIX, p. 87, Kühn), a list of obsolete words or words that are used with a peculiar meaning in Hippocrates' Corpus. According to the glossa, the name might derive from Brauron, located on the eastern coast of Attica (*Ibid*., Loeb vol. V, p. 58). Instead of mentioning its alleged susceptibility to ἐρυσίβη, this source includes Achillean barley in a list of remedies against high fever (Hippocrates, *Diseases*, III, 17). A standard measure of one cotyle of Achillean barley freed of the chaff and boiled in water was regarded as a valuable *rophema* (decoction) with cooling properties, while a richer mixture was obtained by grinding half a cotyle each of barley, white raisins, white chick-peas and safflower and adding celery, mint and coriander.

Among the reasons mentioned for the lower resilience to ἐρυσίβη of barley compared with wheat are not only a higher erectness and a shorter distance of the leaf to the ear, but also a less complete hulledness and a lower softness (closer structure) (Theophrastus, *C.P.*, IV. 14.1–4).

Attention is given in the classical sources to possible remedies against fungal diseases. Among the most common are the treatment of seeds for sowing and the practice of fumigation. The treatment of seeds with vegetal, animal and mineral substances was considered effective also against insects (Columella, *R.R.,* II, 10); the steeping of seeds in a mixture of amurca, a sub-product of olive pressing, and soda or wine is recommended in order to reduce the infection in both the seeds and the roots of germinating seedlings (Plinius, *N.H*., XVIII, 45). Fumigations consisted of making bonfires of trimmings or heaps of chaff and weeds and up-rooted shrubs around the vineyards and fields. The resulting smoke was believed to disperse the disease (*Ibid.,* 293). A remedy less commonly adopted seems to have been the setting on the ground of laurel branches, which was believed to make the infection pass from the fields into the laurel foliage (Plinius, *N.H*., XVIII, 161).

The sources also provide recommendations about how to prepare the threshing floor in order to reduce pest attacks. The desirability of a solid subsurface (Cato, CXXIX) that is levelled and covered with amurca is stressed in order to avoid the formation of cracks and, consequently, the loss of grains and the retention of water. Varro (*R.R.,* LI) states that this treatment was also effective against weeds, ants and moles. However, no explicit mention is made of the potential reduction in plant disease propagation.

The bible contains seven mentions of a plant disease. In each case, the disease can be argued to have been Covered smut. The original Hebrew text uses the noun שדפון (Shdafan), which can be translated as "blight" or "blasting". The root of this word is probably the adjective שדוף (Shadaf) meaning shrivelled or dried up. Based on this root, the word שדפון could refer to the burned appearance of grain infected by Covered smut, but it could also refer to the literal burning of the grain by an extremely hot wind that blows from the Arabian desert, known as *sharav* (Hebrew) or *chamsin* (Arabic), which causes humidity to drop and which literally blasts the grain. This weather phenomenon occurs mostly in spring (May–mid-Jun.) and autumn (Sept.–Oct.) and brings in so much dust that the sky becomes yellowish and hazy for as long as the wind lasts, approximately two to five days. It is interesting to note that this hot wind was able to kill chickens and destroy pasture land, but might also have hastened the ripening of grain, which may have resulted in increased productivity. The Dutch Statenvertaling translates שדפון as "brandkoren" (translation: fire cereal), and a more recent Dutch translation uses the word "korenbrand". *Ustilago Hordei* is today commonly known as *korenbrand* in Dutch. The Dutch translations thus certainly leave room for an explanation of the phenomenon as being some kind of fungus. The English King James translation, on the other hand, which translates the word שדפון as "blasting", has provoked discussion, because this word, like the word שדפון in the original Hebrew, can be interpreted in two ways.

4 Archaeobotanical evidence

4.1 Egypt

Evidence for rachis fragments infected by Covered smut was first discovered among grains from two Roman settlements in the Eastern Desert, namely, Berenike, a harbour located south of Ras Banas, and Shenshef, a mountainous site of which the specific function is still obscure. Both sites were excavated by Leiden University (directed by W.Z. Wendrich) and the University of Delaware (directed by S.E. Sidebotham) between 1994 and 2001. Among the desiccated rachis fragments of Barley were several specimens with a distinctive deformation of the nodes (fig. 1). The asymmetrical hulled grain kernels and the morphology of the non-infected rachis fragments indicate that we are dealing with hulled 6-row Barley.

The infected rachis fragments were found in samples dating to the 1st–2nd, 4th–5th and 5th–6th centuries AD (table 1). The barley from Berenike shows a lower rate of infection than that from Shenshef (0.3–1.3% vs. 11.7%). It has been suggested that barley that was already partly infected with Covered smut was imported from the Nile valley (Cappers 2006).

Subsequently, a re-examination of the subfossil plant remains present in the Dokki Agricultural Museum in Cairo offered the opportunity to check for possible infections in barley from a variety of locations and periods. A total of 442

Figure 1 Desiccated rachis fragments of hulled 6-row Barley (*Hordeum vulgare* ssp. *vulgare*) from Roman Berenike, Egypt, infected by Covered smut.

	BE 1st–2nd	BE 4th–5th	BE 5th	BE 5th–6th	SS 4th–5th
Grain kernels	4 697	267	210	106	206
Rachis nodes (normal)	4 525	1 781	2371	155	769
Rachis nodes (infected)	4	12	63	-	43

Table 1 Breakdown of infected grain kernels and rachis nodes from trash deposits at Berenike (BE) and Shenshef (SS), Egypt, in early and late Roman times (centuries AD).

samples have been checked, including 62 samples containing barley (Cappers and Hamdy 2007).

The re-examination of these samples yielded two samples that contained fragments infected with Covered smut, both originating from Roman Karanis, located in the Fayum. The samples were taken during the excavations performed by the University of Michigan in 1924–1935 and were donated to the museum in 1935 by the Antiquity Department.

Sample 274 consisted of spikelets and a few rachis fragments of hulled 6-Row barley. Other plants present include a grain kernel and rachis fragment of Hard wheat (*T. turgidum* ssp. *durum*); fruit (fragments) of *Raphanus raphanistrum*, *Sinapis* cf. *arvensis*, *Galium aparine*, *Malva nicaeensis*/*sylvestris*, cf. Fabaceae; and a seed of *Lotus*.

Sample 2057 consisted primarily of straw and spikelets of hulled 6-Row barley. In this sample, only a few other plants were present, namely, a fruit of *Galium aparine* and *Zygophyllum* cf. *coccineum* and a seed of *Convolvulus arvensis*.

Other evidence for infected hulled 6-Row barley, discovered by Van der Veen (2011) comes from both the Roman and Islamic ports at Quseir Al-Qadim, located along the Red Sea coast, which were excavated between 1999 and 2003 by the University of Southampton (directed by D. Peacock and L. Blue). Small numbers of infected rachis fragments, preserved by desiccation, were found in samples dated to the Roman period (1st–early 3rd c.; infection rate 0.5%); Islamic period (11th–13th c.; infection rate 7.9%); and Late Islamic period (14th–15th c.; infection rate 3.3%).

4.2 Jordan

Several small fragments of charred rachis fragments of Barley were recently retrieved during the processing of samples from Dayr Alla that were collected in 1996 (Van Doorn, 2011;

Figure 2 Charred rachis fragments of hulled 6-row Barley (*Hordeum vulgare* ssp. *vulgare*) from Iron Age Dayr Alla, Jordan, infected with Covered smut.

Neef *et al*. 2012). A few infected fragments were found in a sample collected near a jar. This jar seems to have been filled with a mixture of flowers from the Rue family (Rutaceae) and seeds from the Cabbage family (Brassicaceae) and of Fenugreek (*Trigonella foenum-graecum*). It is suggested that a spice mixture was stored in the jar (Neef, 1989). It is not clear whether the few grain kernels of Barley (*Hordeum*) and the infected rachis fragments were part of this spice mixture originally or whether they became mixed in afterwards.

Some other infected specimens were found in one of the samples that were taken from a large concentration of burnt seeds present on the floor of one of the rooms of a two-room mud brick building. The house belongs to habitation phase VII (c. 700–650 calBC) and was destroyed by a fire as a result of which the roof, consisting of wooden beams and reed culms, collapsed and fell down.

It has been estimated that some 50–80 kg of seeds covered the floor. Almost 6 kg has been subsampled in four large samples for archaeobotanical analysis. The majority of the burnt material consisted of spikelets of hulled 6-Row barley (*Hordeum vulgare* ssp. *vulgare*). The barley was stored after threshing, as could be inferred from the irregular orientation of the individual spikelets. Other crop plants that were present, though only in small numbers, are emmer (*Triticum turgidum* ssp. *dicoccon*), Hard wheat (*T. turgidum* ssp. *durum*), represented by a single grain kernel and rachis fragment; and flax (*Linum usitatissimum*). In addition, some wild plants were found, most of them being arable weeds, namely, *Hippocrepis*, *Lolium remotum/temulentum*, *Avena*, *Asphodelus*, *Scorpiurus*, *Phalaris paradoxa*, *Bolboschoenus glaucus*, *Convolvulus arvensis*, *Hordeum* (wild species) and some diaspores that have not yet been identified beyond the family level (Poaceae, Apiaceae and Rutaceae).

Only a few rachis fragments of barley were found among the thousands of spikelets, and all of them proved to be infected with Covered smut (fig. 2). The infected fragments consist of the rachis and the lower part of the infected spikelets. The black spore mass is still present within the membranous envelope. That we are dealing with infected rachis fragments only became clear when larger fragments, showing the twisted rachis, were found. Infected rachis fragments consisting of a single rachis node were only recognized during a second inspection of the indeterminate specimens.

So far, records of infected rachis fragments from archaeological contexts are rare. This is probably due to deformation of the rachis fragments, so that they are no longer recognized as such. We hope that through the documentation of desiccated and charred specimens of infected rachis fragments this grain disease will be recognized more often. In this way, a better picture of the spread of this fungal disease and its possible impact on yield loss will be obtained.

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