TRICHODERMA VIRIDE PERS. EX FRIES, AND NOTES ON *HYPOCREA*

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(With 5 Text-figures)

Introduction and historical summary

With the increased interest in *Trichoderma* in recent years, and the description of several new species, the difficulty in specific identification has increased. My views are here presented on the taxonomy of the forms commonly encountered. I am very grateful to E. W. Mason of this Institute for providing cultures, specimens, and sound advice, to Miss E. I. McLennan of the University of Melbourne for sending her notes on *Trichoderma* and *Hypocrea* (from which quotations are given below), and to M. I. Timonin of Ottawa for many cultures and for assistance since 1932 in the study of *Trichoderma*.

Persoon in 1794 founded the genus *Trichoderma*. *T. viride* was described briefly as the first species, with *Pyrenium lignorum* var. *vulgare* Tode, 1790, listed as a synonym. Some confusion in the nomenclature of this species arose during the next few years, but Fries (1829, p. 215, with citations of literature) reduced to synonymy the names he considered to belong to *Trichoderma viride* Pers. ex Fr. This is the type species of the genus. For more than seventy years after Fries, only the one green species was definitely recognized.

It is true that from time to time before Fries and up to 1900 other fungi were described briefly as species of *Trichoderma*, but none of these has yet been verified as a *Trichoderma* distinct from *T. viride*. As Saccardo remarked in compiling thirteen names in *Trichoderma* in the *Sylloge* (1886), all but *T. viride* were doubtful because the method of spore formation was unknown for the twelve others. Only *T. album* Preuss (which Lindau (1904) considered certainly not to belong to the genus) need be mentioned here, for the name has been used for certain cultures from soil, as mentioned below.

Fries and other early mycologists interpreted *Trichoderma* as a Gasteromycete, but Tulasne in 1860 found that it was one of the Fungi imperfecti. The brothers Tulasne (1865) made the brilliant observation that *T. viride* was connected with *Hypocrea rufa*, and illustrated beautifully the conidiophores with phialides surmounted by small

heads of conidia, and did not overlook the chlamydospores. The conidia were described as spherical or broadly ovate, smooth, "hardly exceeding 3·5 μ in the greater diameter, and growing solitary, or shortly moniliform-concatenate, or even fasciculate".

Harz (1871) presented an emended description of the genus *Trichoderma*, with all the emphasis on microscopic characters, and

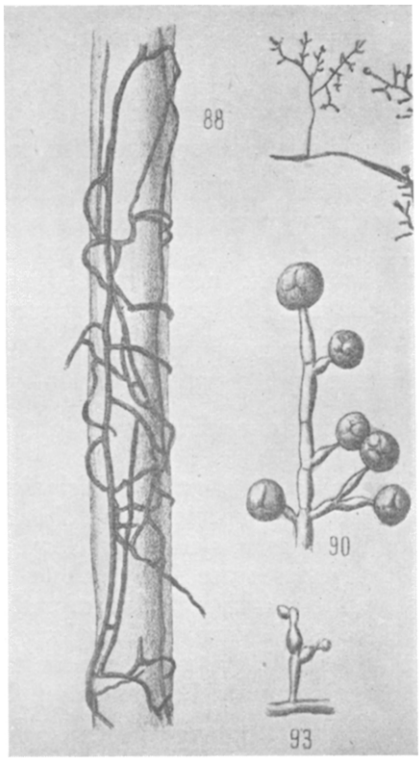

Fig. 1. From Vuillemin (1887), showing (no. 88) the attack of *Trichoderma* on a hypha of *Mucor*.

included only the one species. His figures are good, but Tulasne's show better the usual form of phialide and conidiophore. Harz revived Tode's name, and made the new combination *T. lignorum* (Tode) Harz. Although this name was accepted by Saccardo and many subsequent workers, it is invalid under the present International Rules.

Vuillemin (1887) used the name *Acrostalagmus viridis*, noted the odour of cultures as "pénétrante, légèrement camphrée", and presented good figures, including the attack of *Trichoderma viride* on

a hypha of *Mucor* (Fig. 1). He reported pycnidia, but these must have belonged to another fungus: his figures suggest *Phoma glomerata* (Corda) Wollenw. & Hochapfel (*Coniothecium scabrum* McAlpine).

Brefeld (1891) proved by pure cultures that the ascospores of *Hypocrea rufa* developed *Trichoderma viride* as the conidial stage, thus verifying Tulasne's observations. Brefeld also reported on the conidial stage of *Hypocrea gelatinosa* (see below).

Oudemans in 1902 described *Trichoderma Koningi* from soil. This name also has been transferred to *Acrostalagmus* (Duché & Heim, 1931). Since 1902, *Trichoderma Koningi* has appeared in the literature about equally with *T. viride*. Lindau (1904) listed as doubtful all names except *T. viride* and *T. Koningi*. Bainier (1906) gave notes and figures of his interpretation of the two species. He also transferred *Pachybasium hamatum* (Bonord.) Sacc. to *Trichoderma*, and described as new *T. minutum*, another *Pachybasium*. Bainier has not been followed as regards *Pachybasium*.

Cook & Taubenhaus (1911) compared *Trichoderma Koningi* with *T. lignorum* (both determinations by Thaxter), and reported certain apparent differences. C. N. Jensen (1912) described briefly the appearance of cultures attributed to the two species. Miss Dale (1912, 1914) reported from soil *T. Koningi*, *T. lignorum* with echinulate spores, and revived the name *T. album* for a white form which, to judge from her figures, was not a *Trichoderma*.

Goddard (1913) described as new *T. nigrovirens*, which he said was a common fungus in soil. Gilman & Abbott (1927) considered that it may have been *T. viride*, but Goddard's description and figures leave one in doubt.

Waksman (1916) reported from soil *Trichoderma* strains I–V, with brief descriptions of cultural characters and morphology: I was interpreted as *T. Koningi*, II as *T. lignorum*; III, IV, and V were not named. Waksman also included "*T. album* Preuss" with spores $2{\cdot}5–2{\cdot}9 \times 1{\cdot}8–2{\cdot}1\ \mu$, in heads on lateral conidiophores.

Abbott (1926) studied seven isolations of *Trichoderma* from soil, which "constitute a series of intergrading forms, which show sufficient cultural differences to be considered as separate species when observed individually", but he decided they could be placed as four species: *T. lignorum*, *T. Koningi*, and *T. glaucum* n.sp., for which cultural and morphological characters are given, and the fourth species not named. Abbott's conclusions were no doubt reasonable at the time, when there was less information available regarding soil fungi, and the appearance of fungi in culture: but one cannot help wondering how he would have placed seven hundred isolations of *Trichoderma*.

Other names and literature are included below.

NOTES ON CERTAIN SPECIMENS AND CULTURES

Persoon's herbarium contains six specimens marked *Trichoderma viride*. These represent his concept of the species, and one of them must be the lectotype of the genus and species. Thanks to the Director of the Rijksherbarium at Leiden, I was given the privilege of examining these specimens. Six slides showing microscopic characters are filed at the Imperial Mycological Institute.

Rijksherb. no. 148–1 is marked in Persoon's handwriting "*Trichoderma viride* P. (Prope Pariosos)". It consists of a piece of rough bark 5 × 3 cm. bearing about a score of tufts of the *Trichoderma* in the mature condition, i.e. each tuft (0·5–1·5 mm. in diam.) is dark olive-green, and under a binocular microscope is seen to consist of a mass of dark spores and a few projecting hyphae. A permanent mount was made, which shows the spores to be now pale greenish brown, globoid, 2·5–4 μ, or ovoid, 3·5–5 × 2·5–3·5 μ, surface (as seen with oil immersion) slightly roughened; many clusters of spores still present, and a few typical phialides were seen.

Rijksherb. 148–2 is marked, in Persoon's handwriting, only "*Trichoderma viride*". It consists of a piece of bark 55 × 30 mm., evidently from a kind of tree different from that of 148–1. It bears a few tufts of spores so dark that they hardly look like *T. viride* under a lens; but under a binocular the tufts appear more typical. A mount of spores showed them to be mostly oval, 3–4 × 2·5–3 μ, sometimes globoid, 3–3·5 μ, slightly roughened, often in clusters or "heads".

Rijksherb. 148–3 is marked "*Trichoderma viride*", not in Persoon's handwriting, and in another hand "Hb. Nees v. Esenb." It consists of a bit of smooth bark 25 × 10 mm., with three or four typical tufts of *Trichoderma*. The spores are mostly globose, 2·5–3·5 μ, slightly rough.

Rijksherb. 148–4 is marked by Persoon "*Trichoderma viride* var. *aeruginosum*. Saltem formam magis regularem habet et magis compactum et tomentosum est." It consists of two pieces of bark from a branch or sapling, one mossy, 8 × 1–2 cm., one smooth, 6 × 1·5 cm. The former bears several large tufts 2–5 mm. in diam., or larger by confluence, rather young with a white margin. The smaller piece bears smaller and more mature tufts. Spores from the mossy bark are oval to subglobose, 3–4 × 3–3·5 μ, nearly smooth.

Rijksherb. 148–5 is marked by Persoon "*Trichoderma viride* var. *aeruginosa*". It consists of four pieces of bark bearing numerous tufts of what is apparently young *Trichoderma* still white, but with a suggestion of green colour at the centre of many tufts. A slide was made, and some globoid to ovoid spores, and a few typical phialides of *Trichoderma* were found.

Rijksherb. 148–6 is marked in an unknown hand "*Trichoderma viride* var. *verruginosa*. Herb. Pers. Doublette." What is obviously meant is "var. *aeruginosa*". The specimen consists of several bits of bark with *Trichoderma*, apparently like no. 148–4. The fungus was not studied microscopically.

No. 1 or no. 2 must be chosen as the type specimen, and it seems best to designate no. 1 as lectotype. The examination of Persoon's specimens made it clear that the present-day concept of *Trichoderma viride* is correct, i.e. the same as that of Persoon. Considerable variation in spore characters is evident in his specimens.

Since the time of Persoon thousands of specimens have been filed away in herbaria. Kew Herb. has about sixty specimens in the *T. viride* folder, which extend the idea of the species from Persoon to the

present time. A "random sample" of these specimens was examined for important spore characters:

(1) Collected about 1800, marked "*Trichoderma aeruginosum* Pers. in litt., *Mucor lignifragus* Bull." Spores subglobose, 3–4 (5) μ, or oval and *c.* $4 \times 3\,\mu$, slightly rough.
(2) Marked "*Botrytis lignifraga* Decand., Sept. 1819". Spores $4–5 \times 3–3{\cdot}5\,\mu$, very slightly rough.
(3) Germany, August 1831. Spores $3–4 \times 2–2{\cdot}5\,\mu$, smooth or nearly so.
(4) On *Polyporus squamosus*, England, 2 October 1837. Spores globoid or broadly oval, 2·5–3·5 (4) μ, apparently smooth.
(5) Nilgiris, India (*c.* 1860?). Spores oval, $2{\cdot}5–3{\cdot}5 \times 2–2{\cdot}5\,\mu$, very slightly rough.
(6) Swan River, Australia, Herb. Berk. Spores oval, $4–5 \times 3–4\,\mu$, slightly rough.
(7) Ellis and Everhart, North American Fungi 3077, collected 1893. Spores $3–3{\cdot}5 \times 2{\cdot}5–3\,\mu$.

Trichoderma viride was very common on piles of branches (mostly *Betula*) at Oxshott, Surrey, in October 1938. Many of the colonies had developed young stromata of *Hypocrea rufa*. Collections were studied in the field and laboratory, and in transfers to culture media. The characters of the spores of a few were as follows:

(1) On old *Polyporus betulinus*: spores mostly globoid, $3–3{\cdot}5\,\mu$, but some 4×3 to $5{\cdot}5 \times 4\,\mu$; slightly rough.
(2) On bark of *Betula*: spores mostly globoid, $3–5\,\mu$; a few ovoid; slightly rough.
(3) Also on *Betula*: spores 3–5 (6) $\times 3–4\,\mu$, nearly smooth.
(4) Cultures of *Trichoderma viride* from old *Polyporus betulinus*: spores oval or pyriform, $3–5 \times 2{\cdot}5–3{\cdot}5\,\mu$, becoming definitely roughened. Some cultures were fluffy, some with appressed growth.
(5) Culture from *Betula*: spores globoid, $3{\cdot}5–4\,\mu$, slightly rough.

Since the studies of Tulasne and Brefeld it has been accepted that the perfect stage of *Trichoderma viride* is *Hypocrea rufa*. *Trichoderma viride* can therefore be obtained in culture from ascospores, or from surface-sterilized stromata, of *Hypocrea rufa*.

Mature *Hypocrea rufa* on *Corylus*, Ranmore Common, Surrey, collected 8 October 1933, Herb. E. W. Mason no. 1283, was used in October 1933 by E. W. Mason to obtain single ascospore cultures. After some study of the cultures in the laboratory in Kew, transfers were sent to Dr McLennan, and other cultures in test-tubes were placed in a box in the laboratory. Exactly five years later (October 1938) melted agar was poured into one dried tube, and in a few days the culture was growing again. This revived culture has served the writer as a "test culture" as described below. The following notes on the mono-ascospore culture, which were made by Dr McLennan about 1933, are those of an independent and unbiased observer:

Malt agar, room temperature: mycelium hyaline, appressed; after four days some erect whitish mycelium is present and after six days small white tufts, a few of which are turning yellowish green; after nine days the tufts are coloured dark American green with some baryta yellow (Ridgway) and spores are copiously present.... Oatmeal agar: sporing tufts scattered, so that a flaky or flocculent appearance results; tufts become dull blackish green, in some cases rimmed with white; a few

are pinard yellow....Gelatine, 20°, not liquefied in first week; sporing areas whitish and colonial buff at first, then citrine to ecru olive, and later a buffy olive. After 28 days a buckthorn brown coloration is present in the medium; this deepens to dark brown....Conidiophores arise singly or irregularly grouped, length variable, simple or branched,...[bearing phialides] singly, oppositely or occasionally in whorls; phialides 6–10 μ long, 2·4–3·5 μ wide....Conidia pale green, oval, 2·5–3 × 2–2·5 μ; borne on the tips of the phialides in small heads held together by mucilage.

The single-ascospore culture revived after five years produced cultures which lacked the vigour of the original, and large tufts were no longer produced, but instead a very large number of small tufts giving a flaky appearance; the spore production was less abundant, so that the cultures were first white then a pale greyish yellowish green. The conidiophores, phialides and conidia were as recorded by Dr McLennan, except that some of the conidia reach 4 μ in length, and were measured as 2·5–4 × 2–2·5 μ. Some of the roundish heads of spores are persistent, and about 10–13 μ in diameter.

Although stromata of *Hypocrea rufa* developed in the open in abundance during late October and November 1938, they failed to mature and none was found from which ascospore cultures could be made. However, it was easy to obtain cultures of *Trichoderma viride* by taking the rufous stromata of *Hypocrea rufa*, free from overgrowth of the *Trichoderma* stage, sterilizing the surface in a solution of mercuric chloride, then crushing in sterile water and streaking out the interior of the stroma on agar. Pure cultures of *T. viride* were thus invariably obtained. These isolations varied considerably in cultural characters, but the only noticeable difference from most (but not all) cultures isolated from the *Trichoderma* stage on wood, or received from soil, etc., was a more fluffy growth of aerial mycelium that remained white for a long time, sometimes a month or two in test-tubes. But all cultures were found finally to produce the typical green or yellowish (especially in the isolation listed below as no. 3) tufts of sporulating hyphae with typical phialides and conidiophores of *T. viride*. The spore characters of cultures from a half-dozen different stromata were as follows:

(1) Spores globoid to ovoid, 2·5–3·5 μ, nearly smooth.
(2) Spores nearly all globoid 3–3·5 μ, slightly rough.
(3) Spores globoid, 3–4 μ, or oval, *c.* 4 × 3 μ, slightly rough.
(4) Spores mostly oval, 3–4 (5) × 3–3·5 μ, becoming rough.
(5) Spores mostly ovoid, *c.* 4 × 3 μ, very slightly rough.
(6) Spores 3–5 × 3–4 μ, almost smooth.

The following cultures were obtained from the collection maintained at the Centraalbureau at Baarn:

"*T. lignorum* from Richards." This culture produced usually rather small tufts, green or sometimes yellowish, with the usual phialides, and spores globoid or broadly ovoid, apparently smooth, mostly 2·5–3 μ.

"*T. Koningi*, C.B.S. strain." Spores apparently smooth, mostly 3–4 × 2–2·5 μ. Tufts conspicuous on certain media; on other media the fungus resembles closely macroscopically, and microscopically, the cultures mentioned above as revived from the mono-ascospore culture of *Hypocrea rufa*.

"*T. Koningi* from Szilvinyi." Cultures very similar to the preceding, and to those described by Dr McLennan for *T. viride* from *Hypocrea rufa*. Spores smooth, 3–4 (5) × 2·5–3 μ.

"*T. Narcissi* Tochianai & Shimada" (1931, first described by them in 1930 as *Sporotrichum Narcissi*). The cultures agree with many of those of *Trichoderma viride* obtained from wood, *Hypocrea rufa*, and soil. On Dox agar the tufts tended to remain yellowish green. The spores are globoid or broadly ovoid, slightly roughened, 3–4 (5) μ in diam. The figures of Tochinai and Shimada suggest *Trichoderma viride*, which is known to be sometimes semi-parasitic on higher plants including *Narcissus* (Gregory, 1932, W. C. Moore in litt.).

"*T. Nunbergii* Szilvinyi", 1932, from the feeding galleries of an insect in a forest. Although Szilvinyi designated this as a new species largely on the basis of its cultural characters as compared with those recorded by other workers (especially Abbott, 1926) for *Trichoderma lignorum*, *T. Koningi* and *T. glaucum*, in my view four cultures, not four species, were described. Nothing distinctive was seen in the macroscopic appearance of *T. Nunbergii* in culture. The spores are commonly 3–4 × 2–2·5 μ, smooth or slightly rough; sometimes they may be more elongate (up to 6 μ long) and may show evidence of a faint refractive guttule near each end of the spore; more elongate spores of other cultures also may show guttules. The phialides are as illustrated by Szilvinyi, and like those of *T. viride*. Chlamydospores are common.

"*Eidamia viridescens* Horne and Williamson", 1923. This has been considered by many workers to be a synonym of *Trichoderma viride* (e.g. Gregory, 1932, p. 482) but Asthana (1936) used the name "*T. viridescens*". Horne & Williamson (1923) described the fungus, and especially the cultural characters, very carefully. They were misled as to genus by a misinterpretation of the chlamydospores. I found the culture to agree with *T. viride*.

Comments and observations

It is already evident that I find the variability in morphology and in cultural characters of various isolations of *Trichoderma viride* to be such as to suggest that the names *T. Koningi* and *T. Narcissi* are synonyms of *T. viride*. The cultural characters have not been described in detail, except for the notes of Dr McLennan, which indicate the variability that may be found in a culture from one ascospore. Many

workers have described characters of cultures often probably derived from a single spore of a *Trichoderma*, and assumed them to represent a species. Milburn in 1904 described and illustrated the variation of *T. viride* in culture, and made the illuminating discovery that his culture was green on acid media, but yellow on alkaline.

I have studied the cultural and morphological characters of many scores of isolations of *Trichoderma*, and for a long time I tried to place them under the usual specific names. Bisby (with Timonin & James, 1933, 1935) reported on 337 isolations from soil in Manitoba, where this genus was found to constitute 7 % of the fungi isolated from soil. A few cultures were called *T. glaucum* and *T. album*, but 82 % were called either *T. lignorum* or *T. Koningi*. It soon became evident that all the supposed differences between these species were subject to such fluctuation that only one criterion could be made to suffice for separation of the ordinary green cultures: if the spores were mostly globoid, the culture was called *T. lignorum*; if mostly ovoid, *T. Koningi*. Even some of the "*T. album*" cultures were noted to become green on occasion. Bisby *et al.* (1935) finally expressed themselves as in agreement with H. L. Jensen (1931), who wrote of his cultures of *Trichoderma*: "Only once was a white strain found; all others were green, abundantly sporulating forms, mostly corresponding to the species *Koningi* and *lignorum*, but so variable and with so many transition forms that a sharp distinction was impossible."

Many cultures of green *Trichoderma* have been examined at the Imperial Mycological Institute; they came from various parts of the world, but were like those examined in Manitoba. Finally, in September 1938, Mr Timonin sent thirteen cultures chosen to represent variations in morphology of spores, phialides, conidiophores and tufts and in cultural characters. Mr Timonin's numbers, the source of his cultures, and his measurements of spores are as follows ("rhizosphere" refers to the layer of soil around the root of a plant):

(1)	Soil, Ottawa, Canada	$3{\cdot}5 \times 2{\cdot}5$–$3\,\mu$.
(2)	Rhizosphere, Ottawa	globoid, $2{\cdot}5$–$3\,\mu$.
(3)	,, ,,	$3{\cdot}5$–$5 \times 2{\cdot}5$–$3{\cdot}5\,\mu$.
(4)	,, ,,	$3{\cdot}3$–$3{\cdot}5 \times 2\,\mu$.
(5)	,, ,,	globoid 2–$3{\cdot}5\,\mu$.
(6)	,, ,,	globoid 2–$3{\cdot}5\,\mu$.
(7)	,, ,,	$3{\cdot}5$–$5 \times 2{\cdot}5$–$3\,\mu$.
(8)	,, ,,	4–$4{\cdot}5 \times 2$–$3\,\mu$.
(07)	From Dr Weindling, U.S.A.	globoid $3{\cdot}3$–$5\,\mu$.
(28)	Cotton roots, Texas, U.S.A.	globoid $2{\cdot}5$–$3{\cdot}5\,\mu$.
(58)	,, ,, ,, ,,	globoid $2{\cdot}5$–$3{\cdot}5\,\mu$.
(67)	,, ,, ,, ,,	$3{\cdot}3$–$4{\cdot}5 \times 2{\cdot}5$–$3\,\mu$.
(68)	,, ,, ,, ,,	globoid 3–$3{\cdot}5\,\mu$.

Tests were made with these cultures in comparison with:

T. The "test culture" from a single ascospore of *Hypocrea rufa*.
T 6. A "test culture" from stroma 6 (see above) of *Hypocrea rufa*.
L. *Trichoderma lignorum* from Richards via C.B.S.

KC. *Trichoderma Koningi*, C.B.S. culture.
KS. *Trichoderma Koningi*, from Szilvinyi via C.B.S.
Nar. *Trichoderma Narcissi* from Tochinai and Shimada via C.B.S.
Nun. *Trichoderma Nunbergii* from Szilvinyi via C.B.S.
E. *Eidamia viridescens* from Horne and Williamson via C.B.S.
A. *Trichoderma* from Wattle bark, South Africa.
W. *Trichoderma* from mushroom bed, England, isolated by Dr Ware.
G. From *Hypocrea gelatinosa* (see below).
P. From *Hypocrea pulvinata* (see below).

These various isolations (excluding G. and P.) were grown on clear maize-meal agar in Petri plates, four isolations at random on each plate. There were only minor differences in macroscopic appearance, and the hyphae invariably intermingled slightly when two adjacent colonies met, then both stopped growth. Similar inoculations were made upon richer media, including potato dextrose agar and prune agar. On these media the cultures varied more in appearance, made more luxuriant growth and more overgrowth of hyphae at the line of contact between any two mycelia; but showed no evidence of mutual aversion (see Miss Cayley, 1923) or of parasitism. I did not find any basis for specific difference in the cultural characters.

Hyphal fusions have been considered a possible criterion for distinguishing species of fungi (see Buller, 1931; Matsumoto *et al.* 1932). Hyphal fusions occur commonly in a culture of *Trichoderma*, as Buller showed. The occurrence of fusions between hyphae of the various "strains" was investigated by using the technique of Matsumoto *et al.* (1932), by watching the hyphae from spores placed near each other in Petri dishes, and by cutting out strips of agar between two advancing colonies in Petri dishes to force the hyphae to meet on the surface of the glass. Only what seemed clearly to be "true fusions" were accepted. Every possible combination between the twenty-five cultures was not attempted by this tedious process, but first each culture was tested against T. in at least two tests. The following fusions were observed: T. with 1, 3, 4, 6, 8, 07, 28, 58, T 6, L., KC., KS., Nar., E., and A. For one reason or another fusions were not definitely seen between T. and the cultures not mentioned. These were tested with other strains, and fusions were found between T 6 and 2, 5, 28, T., Nun., KC., W., and G.; between KC. and L., 67 and W.; between G. and Nun., T 6, KC., and E.; between 68 and 2; between 8 and 7. Fusions were not found between P. and other cultures tested.

These hyphal fusions support the view, gained from study of the morphological and cultural characters, that the various cultures of *Trichoderma* represent one variable species.

The bases for separating the so-called species just considered will now be examined.

T. Narcissi: no reason given by the authors for considering it

distinct from *T. viride*; no reason is found in the description or in the characters of the culture, and hyphal fusions readily occur with *T. viride*.

T. Nunbergii: supposed cultural differences between this and *T. viride*, *T. Koningi* and *T. glaucum* are given by Szilvinyi, the spores are cited as $3{\cdot}3 \times 2{\cdot}2\,\mu$, and the fungus was found in an insect gallery. None of these points provides a definite basis for a new species. The cultures show spores sometimes unusually elongate (i.e. the fungus might be *T. Koningi* if that be distinct).

The name *T. Koningi* is so firmly entrenched in the literature of thirty-seven years that it requires special attention. Oudemans distinguished it by "elliptic" spores $3–4 \times 2{\cdot}5–3\,\mu$, in heads $8–10\,\mu$, whereas *T. viride* he interpreted as having spores "absolument globuleuses, plus petites, réunies en glomérules dont le diam. ne dépasse pas $5{\cdot}7\,\mu$". Apart from these possible points of difference one finds nothing in his description or figures to exclude *T. viride*. *T. Koningi* had woolly tufts first white then green, and the figures of phialides and chlamydospores would pass excellently for those of *T. viride*. Subsequent workers soon found that the suggested difference in size of spore clusters was imaginary, and the pages preceding this show that there was evidently no significance in the minute differences in shape or size of spores suggested by Oudemans: the ascospore culture from *Hypocrea rufa* has the spores of "*Trichoderma Koningi*", other specimens or cultures of *T. viride* have spores from globose to more "elliptical" than $3–4 \times 2{\cdot}5–3\,\mu$, and the spores may be larger. But a confusing series of other presumed differences has been suggested by subsequent workers, e.g. colonies floccose and conidiophores up to $25\,\mu$ long in *T. Koningi*, colonies tufted and conidiophores up to $70\,\mu$ long in *T. viride*; differences in amount of mucus or shape of phialides. These points hardly need be discussed: the floccose or tufted appearance, the amount of mucus, and even to some extent the shape and length of the phialide, vary with the individual and with the conditions of culture. As for the length of the indefinite conidiophore, it may bear one phialide or ramify through a tuft, so that $10–1000\,\mu$ more accurately expresses the length. On the other hand, Duché & Heim (1931) record globoid spores up to $2{\cdot}5\,\mu$ for what they called *T. Koningi*, Miss Niethammer (1937) roundish spores for *T. Koningi* and ellipsoid for *T. viride*, and Stevenson & Rands (1938) give the spores of *T. Koningi* as $3{\cdot}2–4{\cdot}8 \times 1{\cdot}8–3\,\mu$, of *T. lignorum* as $3{\cdot}8–5 \times 2{\cdot}5–3\,\mu$.

I have found no basis for separating from *T. viride*, even as varieties, *T. Narcissi*, *T. Nunbergii*, and the description of *T. Koningi* and cultures attributed to the name (type material of *T. Koningi*, if kept, I have not seen). Hyphal fusions occur between the various cultures. When all is said, *T. viride* is not even surprisingly variable; the part-spores of its perfect stage are (Petch, 1938) "globose $3{\cdot}5–4{\cdot}5\,\mu$,

or oval, 5–6 × 3·5–4 μ", and one may expect the conidia of a species to vary as much as its ascospores.

I have conscientiously sought other possible criteria for distinguishing species or varieties of *Trichoderma*. There is the point of minutely roughened spores, mentioned for *T. viride* by Miss Dale (1914) and Petch (1938). But higher powers of the microscope show most spores, or might show all, to bear a slightly roughened surface. I have not ascertained the nature of the inconspicuous roughening of the spores (they are usually recorded in the literature as smooth). The roughness might conceivably be due to the slime accumulated in patches, but ether does not remove it.

A few tests were made with cultures on media made alkaline by the addition of dilute potassium hydroxide. Milburn found his culture to be yellow in certain alkaline media. Horne & Williamson (1923) found the growth limits to be *p*H 2 and 8·2. My tests were inconclusive: some strains produced no tufts, some yellowish, some greenish tufts. The striking thing was the retardation of growth; colonies after 13 days were only 10–35 mm. in diameter. Bisby *et al.* (1935, p. 56) report that they failed to isolate *Trichoderma* from an alkaline soil.

The maximum temperature for the various strains of *Trichoderma* was found to be 35 to about 38° C. (see also Bisby *et al.* 1935, p. 63). At temperatures of 20–30° C. growth is rapid, but varies with the individual culture and with the medium.

The great variation in the appearance of cultures can be demonstrated by anyone who will make transfers of one culture to various media, including one with high sugar content and one with an alkaline reaction.

The odour of cultures of *Trichoderma* was recorded by Vuillemin as camphorated and by Horne and Williamson as that of coconut oil. About a dozen people were asked (without a suggestion as to what the odour had been called), and the majority soon said "coconut" or "coconut-cakes". No one had another definite suggestion. This odour is often very marked on certain media (including prune agar and potato-dextrose agar). The six cultures mentioned above as isolated from *Hypocrea rufa* all had the odour at first, but three soon lost it. The cultures of "*Eidamia viridescens*", however, still have the odour after sixteen years in culture. Most isolations from soil lack the odour.

In looking carefully at phialides, it was noted that the spores were sometimes in chains of a few to about a score of spores. This seemed an excellent character for distinguishing species. However, Tulasne, and Horne & Williamson had mentioned but not illustrated spores in chains in *Trichoderma viride*, and a figure by Oudemans seems to show a chain of spores which he did not mention for *T. Koningi*—and careful examination of most of the various cultures of *Trichoderma* showed

the occurrence of some spores in chains, the longer chains occurring with more globoid spores (Fig. 3). The physical conditions requisite for the production of chains were not ascertained, but they developed best under damp conditions. Of course, the spores in *Trichoderma* are

Fig. 2. Semi-diagrammatic figures to show the development of phialides and spores in *Trichoderma*. The young phialide lacks a firm wall, but this develops from below upward. A septum (no doubt perforated by a pore) is formed at the base as the phialide matures. The initial of the first spore is evident early. This spore becomes cut off at the neck of the phialide, and successive young spores are pushed out. The spores usually accumulate in a cluster held together by slime. See Vernon, *Ann. Bot.* XLV, 736, fig. 2, 1931, for development of spores.

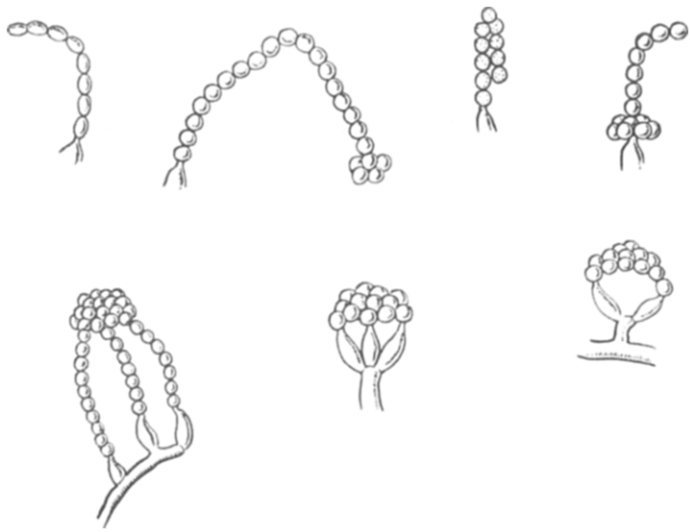

Fig. 3. Sketched from various cultures of *Trichoderma viride*, mostly on agar on slides in a damp chamber. The spores sometimes develop in chains, are roundish or oval, sometimes evidently roughened. The spores from two or more phialides may form a common cluster.

normally formed in heads and upon drying may be held together rather persistently by a slimy substance. Sometimes the heads of spores from two or three phialides may combine. This association of slime with spores is an important character (see Mason, 1937). It precludes the spread of spores by air currents, in the laboratory at least. The tufts of spores of *Trichoderma* are often recorded as

"powdery", but it is a damp powder very different from the dust of a dry-spored fungus such as *Penicillium*.

I believe I have shown that the common green *Trichoderma* constitutes one somewhat variable species, and that mycologists from 1829 to 1902 were right in so considering it. The use of culture media tempted some workers to describe species on cultural and morphologic characteristics that are inadequate when a wide range of specimens is studied.

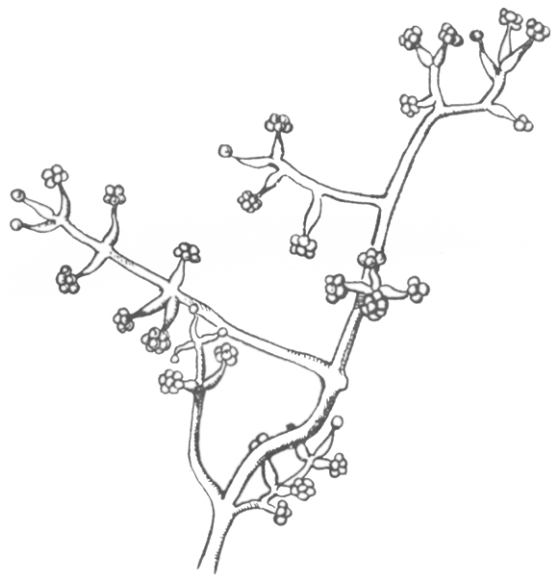

Fig. 4. *Trichoderma viride*, copied from Tulasne, 1865, the right-hand portion of fig. 7, pl. III, vol. III.

Description

The genus *Trichoderma* Pers. ex Fr. is distinguished by considering in conjunction these macroscopic and microscopic characters: tufts or cushions of hyphae normally appear on natural substrata or in artificial culture; the tufts are composed of conidiophores, many spores and some sterile hyphae; conidiophores indefinite, consisting of an unbranched or branched hypha, bearing phialides laterally and terminally; phialides surmounted by heads (rarely by short chains) of slime spores; spores hyaline or brightly coloured under the microscope, one-celled.

Trichoderma viride *Pers.* (1794 *et post*) *ex Fries* (1829)

Fries and others cite several early names as synonyms. I have not attempted to verify these.

Trichoderma lignorum (Tode) Harz (1871).
Acrostalagmus viridis (Pers. ex Fr.) Vuill. (1887).
Trichoderma Koningi Oudem. (1902).
Eidamia viridescens Horne & Williamson (1923).
Sporotrichum Narcissi Tochinai & Shimada (1930).
Trichoderma Narcissi Tochinai & Shimada (1931).
Acrostalagmus Koningi (Oudem.) Duché & Heim (1931).
Trichoderma Nunbergii Szilvinyi (1932).
T. viridescens (Horne & Will.) Asthana (1936).

Spreading vegetative hyphae usually not conspicuous in the field, but in culture they form quickly and grow rapidly into main hyphae about 10–12 μ in diameter, tapering to 2 μ in the smaller branches; septa about 25–100 μ or more apart; these vegetative hyphae usually form a thin, inconspicuous growth on media with little organic food, but usually form a fluffy growth on richer media, white or whitish, medium uncoloured or turned yellowish or brownish, odour of cultures sometimes of coconut. Chlamydospores are formed on natural substrata or artificial culture, sometimes within a day or two in culture, sometimes only after several days, globoid or oval, terminal or intercalary, mostly 7–14 μ in diameter. Tufts in "Hoch-Kultur" first white, commonly 1–5 mm. in diameter, *c.* 1 mm. high, sometimes becoming yellowish, golden or brownish, but usually soon becoming green first within (and surrounded by uncoloured, branched hyphae), then the greater part of the tuft becomes green throughout, with different shades of green corresponding roughly to the varying shades of green leaves. The tufts may sometimes remain white or yellowish, or they may even be almost absent. Conidiophores not sharply distinct from the vegetative hyphae from which they arise, sparingly to abundantly branched, the branches arising at approximately 90° from the parent hypha or portion of conidiophore. Phialides rather distinctive, nine-pin shaped (narrowed at the base and more so above), 8–15 (20) μ long, 2–4 (5) μ wide at the centre, arising singly or opposite or in whorls seldom of more than three phialides, each soon surmounted by a head of about five to thirty or more spores held together by slime; heads often rather persistent, 7–15 μ in diam., subglobose; chains of spores sometimes produced. Conidia arise by the extrusion of protoplasm through the neck of the phialide; each conidium after becoming provided with a firm wall is cut off, and is usually held by surface tension near the apex of the phialide; conidia globoid and 2·5–4 (5, rarely 6) μ in diameter or oval and 3–4 × 2·5–3·5 μ, more rarely sub-elliptic and 4–5 (6) × 2–3 μ, range 2·5–5 (6) × 2–4 (5, rarely 6) μ, pale bright green under the microscope, one-celled, contents granular, wall thin, smooth or commonly marked with inconspicuous roughenings about 0·5 μ apart; on germination the conidia swell to globoid form and 6–9 μ diam., producing hyphae which anastomose readily.

Commonly found fruiting on dead wood or stems, where it often develops into *Hypocrea rufa*; occasionally somewhat parasitic to higher plants in dormant or weakened condition; abundant in the soil, where it is important in decomposing organic material and in parasitizing other fungi; widely distributed over the world.

Fungi related to *Trichoderma viride*

Early mycologists, who considered *Trichoderma* to be a Gasteromycete and who relied largely on macroscopic characters for diagnosis, evidently placed certain Myxomycetes, the sporodochia of fungi, etc., in *Trichoderma*. Recent mycologists, who rely largely on

microscopic characters, have placed *Trichoderma* as *Acrostalagmus* and *Sporotrichum*, and *Pachybasium* as *Trichoderma*. *Pachybasium* is separable because the conidiophores have sterile apices and conspicuous tufts are not formed. *Acrostalagmus* (and *Verticillium* if distinct from *Acrostalagmus*) differs from *Trichoderma* principally in macroscopic characters, but possibly also in the more definitely verticillate arrangement of the phialides. *Sporotrichum* does not have heads of slime-spores.

It is especially noteworthy that *Trichoderma viride* can produce spores in chains, that the spores from more than one phialide often combine into a common cluster, and that, in a saturated atmosphere, the phialides may become more elongate than usual. In these respects relationship with *Gliocladium* is shown.

Other names in *Trichoderma*

The writer would interpret *T. glaucum* Abbott, from the description, as *T. viride* in the not uncommon yellowish condition, which Milburn found could be induced by an alkaline medium. *T. nigro-virens* Goddard seems uncertain, and unless type material can be examined the name might be discarded. The several older and still more doubtful names in *Trichoderma* also require examination of type material if available, or discarding if there is no type. It seems obviously unlikely that species of *Trichoderma* can be present in Europe or North America and have been collected but once.

The only type specimen of a *Trichoderma* in Herb. Kew. is that of *T. lateritio-roseum* Lib. in Cooke, *Grevillea*, VIII, 83, 1880, on rotted potato tubers. An examination of the specimen shows sporodochia; Cooke saw the microconidia but overlooked the macroconidia of *Fusarium* which are also present. The name must be discarded.

I consider *Trichoderma* to be a monotypic genus.

Hypocrea gelatinosa (Tode ex Fr.) Fr. and its conidial stage

Sphaeria gelatinosa Tode was described in 1791 in *Fungi Mecklenb.*, which also includes the "Pyrenium" now called *Trichoderma viride*. Tulasne, 1865, p. 30, states: "*Sphaeria gelatinosa* Tode, which is exactly analogous to *Hypocrea rufa* Fr., seems to have been known to Tode himself as being of a double nature." *Sphaeria gelatinosa* was accepted in the *Systema*, but Fries later transferred it to *Hypocrea*. Seaver (*Mycologia*, II, 58, 1910) made it the type of his genus *Chromocrea*, "distinguished from *Hypocrea* by the colored spores",

Brefeld (1891) reported the conidial stage of *Hypocrea gelatinosa* to be like the *Trichoderma viride* he obtained from ascospores of *Hypocrea rufa*, and states "Fig. 57 kann auch für *H. gelatinosa* gelten". It is true that he gave the conidia from the latter as $5 \times 4\,\mu$, those from *H. rufa* as

globular, 2–2·5 μ, but both measurements could, as he thought, represent *Trichoderma viride*. The conidia of both were greenish. Brefeld mentions a stromatic layer formed in cultures from *Hypocrea gelatinosa*.

Milburn (1904) reported briefly on a culture of *Trichoderma* obtained from what he thought to be *Hypocrea gelatinosa*, and gives only minor differences between it and *Trichoderma viride*.

Vincens (1917) studied what he considered to be *Hypocrea gelatinosa*, particularly the development of asci and perithecia. He found stromata in a wood in late February, pulverulent from the conidial stage, which his illustration shows had phialides and spores like those of *Trichoderma viride*. He found that the spores from a small cluster of phialides united into a common head. He does not report cultures. Vincens states: "Tandis que les coussinets conidifères de l'*Hypocrea rufa* verdissent au moment de la formation des conidies, ceux de l'*H. gelatinosa* ne dépassent jamais une teinte légèrement ochracée tant que les conidies se forment." Stromata placed in a damp chamber in the laboratory developed the conidial stage so abundantly as to arrest the development of the perithecia.

In reality, neither Brefeld, Milburn, nor Vincens found adequate reason for distinguishing the conidial stage of *H. gelatinosa* from that of *H. rufa*. A well-known American mycologist also has remarked recently in a letter that he obtained what appeared to be *Trichoderma viride* in cultures made from *Hypocrea gelatinosa*.

Stromata, appearing typical of *H. gelatinosa*, were collected by Miss K. Sampson (of Aberystwyth) in the autumn of 1933 and sent to E. W. Mason, who made isolations from ascospores. Several cultures of the resulting conidial stage, after preliminary study, were placed in a box in the laboratory. Five years later melted agar medium was poured into one of the dried tubes, and as in the culture from *H. rufa*, the fungus promptly revived.

This conidial stage produced spores 3–4 × 2·5–3·5 μ, and in every respect agrees with *Trichoderma viride*. It was studied very carefully in a search for distinguishing characters, and many of the details regarding *T. viride*, such as the production of spores sometimes in chains, were actually first observed in the culture from *Hypocrea gelatinosa*. It even produces the coconut odour characteristic of many cultures of *Trichoderma viride*, and hyphal fusions readily occur with hyphae from *T. viride*. Older cultures formed the yellow stromatic development recorded by Brefeld—but so did certain cultures isolated from stromata of *Hypocrea rufa*. No perithecia or asci were found in any culture of *H. gelatinosa* or of *Trichoderma viride*. Miss Niethammer (1937) found a perithecium in a culture she called *T. Koningi*, but its identity is doubtful.

The problem involved here, i.e. the formation of the same conidial

stage by two species which some place in separate genera, can only be solved by further study of the *Hypocrea* stages, and more cultures. An examination of the numerous collections in Herb. Kew. suggests that *H. gelatinosa* is only a growth form or mature condition of *H. rufa*. Many specimens at Kew marked *H. gelatinosa*, including Fries Scler. Suec. 304, do not appear to me to be distinguishable from *H. rufa*, nor many marked *H. rufa* from *H. gelatinosa*. Tulasne (1865) says, of the cushions of *H. rufa*, "a very few... become at length dark-aeruginous".

An effort was made to follow to maturity the development of stromata of *H. rufa* during the autumn and winter of 1938–9. Specimens placed in a damp chamber merely produced masses of conidiophores of *Trichoderma*, as Vincens found for *Hypocrea gelatinosa*. If kept saturated, some stromata became gelatinous, but did not mature asci. (Horne & Williamson found that the mycelium of *Trichoderma viride* may become gelatinous.) Specimens left in the wood or placed in a garden reverted to *T. viride*, which the rain gradually washed away.

Hypocrea pulvinata Fuckel and its conidial stage

Hypocrea pulvinata (with several probable synonyms) is commonly found on old *Polyporus betulinus*. Cultures were made by E. W. Mason in 1933 and sent to Miss McLennan, and fresh cultures were made by me from stromata collected at Oxshott in October 1938. The fungus is homothallic, and cultures from a single half-ascospore or single conidium soon produce mature stromata with abundant ascospores.

The conidial stage in culture consists of phialides 25–40 μ long, or often reaching 75 μ or more; they develop singly from the vegetative hyphae or several may come from a single hypha making it an indefinite conidiophore. The shorter phialides are provided with a septum at the base only, the longer ones with an additional septum or two (in which case only the terminal cell should probably be considered the phialide); the phialides soon produce slime-spores at the apex and the spores gather into clusters. The spores are hyaline, one-celled, variable in size, often globular and 3–8 (10) μ in diameter, sometimes pyriform or oval. The fungus makes a thin spreading growth on the culture media used, and soon produces whitish patches or areas of conidial production, followed by the development of roundish or oval yellowish stromata mostly 2–5 mm. wide, often confluent; the stromata in culture are like those in the field.

The conidial stage is probably best referred to *Cephalosporium*. It approaches *Trichoderma* in the formation of patches, but no tufts as definite as those formed by *T. viride* were seen in culture, and the

phialides are also indefinite. I have not observed the conidial stage out-of-doors. Dr McLennan went through the literature of *Cephalosporium*, and suggests that *C. macrocarpum* Corda, described on old *Polyporus*, may represent this conidial stage. Ruhland (1900) figured the conidial stage of *Hypocrea fungicola* (perhaps a synonym of *H. pulvinata*) but did not name it.

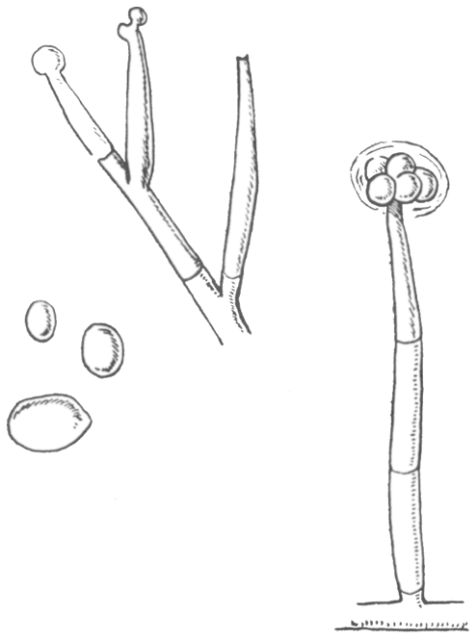

Fig. 5. The conidiophores and conidia of *Hypocrea pulvinata*. The phialides may arise singly, or may develop successively to form indefinite conidiophores. The spores are variable. (Figs. 2–5 redrawn by Mrs G. R. Bisby.)

Transfers of the single-ascospore culture of *H. rufa*, T 6 from a stroma of *H. rufa*, the ascospore culture of *H. gelatinosa*, and a single half-ascospore culture of *H. pulvinata* have been sent to the Centraalbureau at Baarn, the National Collection of Type Cultures in England, and to the American Type Culture Collection. The other cultures mentioned above are being kept (dried) at the Imperial Mycological Institute, but should be available for some time to anyone who wishes to restudy *Trichoderma*.

Summary

Trichoderma viride Pers. ex Fr., the conidial stage of *Hypocrea rufa*, has been known since about 1790. Persoon's specimens, other specimens from herbaria or the field, and cultures from *H. rufa*, soil,

etc., show the fungus to be variable. The most noteworthy variable character is the shape of the conidia, which are subglobose in some isolations, ovoid in others; but the same variation is found in the shape of the ascospores. The name *Trichoderma Koningi*, based principally on the oval shape of the conidia, is considered untenable for various reasons. A single ascospore culture of *Hypocrea rufa* produced conidia as described for *Trichoderma Koningi*. *T. Narcissi* and *T. Nunbergii* do not differ from *T. viride*.

T. viride was obtained from ascospores of a specimen determined as *Hypocrea gelatinosa*. Brefeld and others have reported the same result. It is suggested that *H. gelatinosa* is only mature *H. rufa*.

Hypocrea pulvinata produces a conidial stage considered to belong to *Cephalosporium*. It is homothallic, and readily forms ascospores in culture.

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