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Description of a Distinctive Aflatoxin-Producing Strain of *Aspergillus nomius* that Produces Submerged Sclerotia

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Abstract A new distinctive strain of *Aspergillus nomius* that produces the potent mycotoxins, aflatoxins, is described from pistachio, pecan, and fig orchards in California. Similar to the typical strain of *A. nomius* (as represented by the ex-type), the O strain produced both B and G aflatoxins but not cyclopiazonic acid, had similar conidial ornamentation, and grew poorly at 42°C. Furthermore, previous published DNA sequence supports that the new strain is very closely related to the ex-type of *A. nomius*. However, the O strain differs from the ex-type in several morphological characters. The ex-type was initially described as producing "indeterminate sclerotia" that appear as large (up to 3 mm long) elongated sclerotia on surfaces of media. The O strain produces only small spherical sclerotia (mean diameter <0.3 mm) submerged in the medium. In addition, the O strain has predominantly uniseriate conidial heads, whereas the typical strain of *A. nomius* has predominantly biseriate heads. The O strain colony color on both Czapek solution agar and Czapek yeast extract agar was more yellowish than the ex-type of *A. nomius* and other common aflatoxin-producing fungi. Isolates of the O strain reported here from several orchards represent the first report of *A. nomius* in California.

Keywords *Aspergillus flavus* · *Aspergillus parasiticus* · Cyclopiazonic acid · Fig · Pecan · Pistachio

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Introduction

Aflatoxins are the mycotoxins most widely regulated by governments with the tolerances set extremely low [31]. Among the species of fungi in *Aspergillus* section *Flavi* that produce these potent carcinogens, *Aspergillus flavus* Link and *A. parasiticus* Speare are typically the most common, while *A. nomius* Kurtzman et al. is only occasionally detected. For example, in an extensive study of soil populations of *Aspergillus* section *Flavi* throughout a large area of the USA, it was found that *A. flavus* and *A. parasiticus* were widely distributed but *A. nomius* was only rarely found [16]. In our extensive work with *Aspergillus* species associated with California tree crops, we frequently isolated

A. flavus and *A. parasiticus* but never isolated any fungus matching the description of *A. nomius* [7–9]. Occasionally, however, we isolated a fungus clearly matching the general characteristics of *Aspergillus* section *Flavi*, but it had several morphological and physiological characteristics that did not match previously described species in the section. Genetic analysis has indicated that these unusual isolates are distinct from *A. flavus* and *A. parasiticus* but very similar to the ex-type of *A. nomius* [11, 12]. We decided that the many phenotypic differences between these unusual isolates and the ex-type of *A. nomius* and the importance of aflatoxin production warranted the more detailed description of this new strain of *A. nomius* that is presented in this article.

Materials and Methods

Fungal Isolates Used

For long-term storage, isolates were stored on silica gel at 6°C [30]. The following eight isolates of the O strain (all from California, USA) were used for most experiments: ATCC201127 (pistachio orchard, Madera County), ATCC208927 = NRRL29213 (pecan nut, Fresno County), ATCC208928 = NRRL29212 (washing of pistachio nuts, Madera County), A26 (pecan nut, Fresno County), A27 (pistachio nut, Merced County), A28 (pistachio nut, Madera County), A36 (pistachio orchard soil, Kern County), and A227 (pistachio hull, Madera County). For making comparisons, the following isolates were also used: *A. nomius*, ATCC15546 = NRRL13137 (ex-type) and ATCC96015 = NRRL 6552; *A. flavus*, ATCC16883 = NRRL1957 (ex-type) and A228 (pistachio orchard, Madera County); and *A. parasiticus*, ATCC1018 = NRRL502 (ex-type) and A240 (pistachio orchard, Madera County). In addition, for certain experiments either *A. flavus* S strain isolate ATCC MYA-383 = AF42 and *A. flavus* AF13 (wild type of ATCC 96044) [3] were used or additional isolates of *A. flavus* and *A. parasiticus* that originated from California nut orchards (maintained in the authors' culture collection at the Kearney Agricultural Center) were used. Living cultures of ATCC201127, ATCC208927, and ATCC208928 have been deposited in the American Type Culture Collection, Rockville, MD, USA. The isolates with numbers preceded by only an 'A' are maintained in the authors' culture collection at the Kearney Agricultural Center, Parlier, CA.

Characteristics of Aflatoxin-Producing Species on Various Media

For characterizing the O strain, we used the media and growth conditions of Raper and Fennell [28], Klich and Pitt [20], and Klich [17]. The following five media were used: Difco Czapek solution agar (CZ), Czapek yeast agar (CYA), malt extract agar (MEA), Czapek yeast extract agar with 20% sucrose (CY20S), and Aspergillus flavus and parasiticus agar (AFPA). The formulas for CYA, MEA, and CY20S are given in both Klich [17] and Klich and Pitt [20]. AFPA, a selective medium for *A. flavus* and *A. parasiticus* [26], was used to see whether the O strain would produce the orange reverse characteristic of other aflatoxin-producing fungi. For consistent colony color [29], 1 ml of a copper solution (0.5 g $CuSO_4 \cdot 5H_2O$, 1.0 g $ZnSO_4 \cdot 7H_2O$ in 100 ml deionized water) was added to each liter of CZ. Microscopic characteristics were evaluated on CZ and CYA after incubating cultures at 25°C for 7 days. The colony color and growth of the colony were determined on CZ, CYA, MEA, and CY20S at 25°C after 7 days. In addition, colonies were grown on CYA at 37°C. The growth of aflatoxin-producing fungi was determined by inoculating the center of plates of CZ (three replicated plates), incubating at 10, 15, 20, 25, 30, 37, and 42°C, and measuring colony diameters after 5, 7, and 14 days. Colony color was evaluated using the Methuen color system [21]. Besides making observations on multiple plates for each isolate, all experiments were repeated at least once.

Production of Sclerotia

Czapek–Dox broth (Difco) was solidified with 2% bacto agar and supplemented with 2 ml per liter Nitsch and Nitsch vitamins (Sigma, St. Louis, MO). After inoculation, plates were incubated at 31°C in a water-jacketed incubator. After 30 days, the number of sclerotia on the surface of and submerged in the medium was counted, and the length of the sclerotia was measured. In order to improve the visibility of the sclerotia, plates were first flooded with 95% ethanol, and the conidia were washed off with water [3].

The time course of sclerotial formation by several isolates of the O strain was determined in independent

tests under the mentioned earlier conditions. The reverses of plates were examined under a stereomicroscope, and sclerotia were enumerated periodically between 5 and 43 days of incubation. Plates were not treated with ethanol, and conidia were not washed off for these tests, which included five replicates and were performed twice.

Production of Mycotoxins

Aflatoxin production in liquid fermentation was compared among the O strain isolates, the ex-type of *A. nomius*, and known aflatoxin-producing isolates of *A. flavus* and *A. parasiticus*. Aflatoxin production was quantified in the medium of Adye and Matales [1] with 3 g/l of NH_4SO_4 as the sole nitrogen source as previously described [5]. Erlenmeyer flasks (250 ml) containing 70 ml of medium were inoculated with approximately 5×10^3 spores/ml. After shake incubation (150 RPM, 31°C, 5 days), 70 ml acetone was added to lyse cells and release aflatoxins from mycelia. Filtrates were passed through Whatman No. 4 paper, combined with an equal volume of water, and extracted twice with 25 ml of methylene chloride. Extracts were filtered through a bed of anhydrous sodium sulfate (~40 g), combined, evaporated to dryness, dissolved in methylene chloride, and separated along with aflatoxin standards by thin-layer chromatography (TLC). Extracts were either diluted or concentrated to permit accurate densitometry, and the aflatoxins were quantified on the TLC plates by scanning densitometry [27].

In another experiment, the O strain and other aflatoxin-producing fungi were tested for mycotoxin production using methods that used agar media and TLC [13, 14]. The fungi (besides the isolates used in other experiments, eight additional isolates of both *A. flavus* and *A. parasiticus* collected from pistachio orchards in California were also used) were grown in glucose yeast agar medium (20 g glucose, 5 g yeast extract, 20 g agar, and 1 l deionized water) and in CYA for aflatoxin and cyclopiazonic acid production, respectively, and the mycotoxins were detected using TLC. For each medium, two culture dishes per isolate were incubated at 25°C for 7 days, and then 4-mm diameter agar plugs were removed from the colony for the and placed at the origin on TLC plates (silica gel G). After adding 20 μl of extraction solvent (chloroform/methanol, 2:1) was placed on each plug to extract the mycotoxins [14], the TLC plates were developed in a solvent mixture of diethyl ether/methanol/water (96:3:1) and of benzene/acetic acid/methanol (90:5:7) for aflatoxins and cyclopiazonic acid respectively. The appearance and R_f of the spots for the mycotoxins were compared with standards (Sigma, Dallas, TX). Each of the four aflatoxins (B1, B2, G1, G2) was visible when present using this method. The experiment was repeated, and the results were combined for statistical analysis.

Aflatoxin production was also tested in pistachio nuts by inoculating the nuts with fungi in section *Flavi*, including isolates ATCC201127 and ATCC208927 of the O strain, and measuring aflatoxins using HPLC. Pistachio nuts were collected from orchards, and the nuts with intact hulls were surface sterilized in 0.5% NaOH for 2 min. The hulls were removed, and nuts without split shells were discarded. The nuts with split shells were placed on sterilized wire racks in plastic containers with water on the bottom (not touching the nuts). The kernels were wounded with a sterile needle (~4 mm deep) and inoculated with 10 μl spore suspension (10^5 conidia/ml in 0.05% Tween 80). Two replicates of ten nuts were inoculated for each isolate and incubated at 30°C for 7 days. Nuts were then stored at −19°C until extracted and analyzed for aflatoxins using HPLC [8]. All four of the aflatoxins (B1, B2, G1, G2) were detectable using this method. The experiment was repeated, and the results combined for statistical analysis.

Results

Macroscopic Characteristics

The O strain differs from the other common aflatoxin-producing fungi in several ways (Table 1). Colony diameters of the O strain after 7 days at 25°C were 52–60 mm, 65–70 + mm, and 62–70+ mm for the commonly used media CYA, CY20S, and MEA respectively. In general, the O strain differed from the other aflatoxin-producing fungi in colony color on both CZ and CYA (Fig. 1). After 7 days at 25°C on CYA, the color of the O strain colonies due to the conidial heads was olive (1–2D–E6–7, according to the Methuen color system [21]) compared to other shades of green for the typical strain of *A. nomius*

Table 1 Summary of characteristics for aflatoxin-producing species in *Aspergillus* section *Flavi*

Characteristic	O strain	*A. nomius*	*A. flavus*	*A. parasiticus*
Colony color on Czapek yeast agar (7 days)	Olive (1E6)[a]	Grayish green (30D6)	Grayish green (30D5)	Grayish green (30E7)
Colony color on Czapek yeast agar (21 days)	Olive brown (4E5)	Grayish green (30E6)	Grayish green (30D6)	Dark green (30F6)
Sclerotium location for media	Submerged	On surface	On surface	On surface
Sclerotium length µm	<500	>500	Variable	>500
Longest stipe height[b] mm	>0.7	>0.7	>0.7	<0.7
Predominant type of head	Uniseriate	Biseriate	Biseriate	Uniseriate
Spore ornamentation	Moderately rough	Moderately rough	Smooth	Rough
Aflatoxins produced	B and G	B and G	B only	B and G

[a] In parentheses are typical colors according to the Methuen system of Kornerup and Wanscher [21]. The first number refers to the hue, the letter to the tone, and the last number to the intensity

[b] Stipe height is the distance from the foot cell to the vesicle of the conidiophore

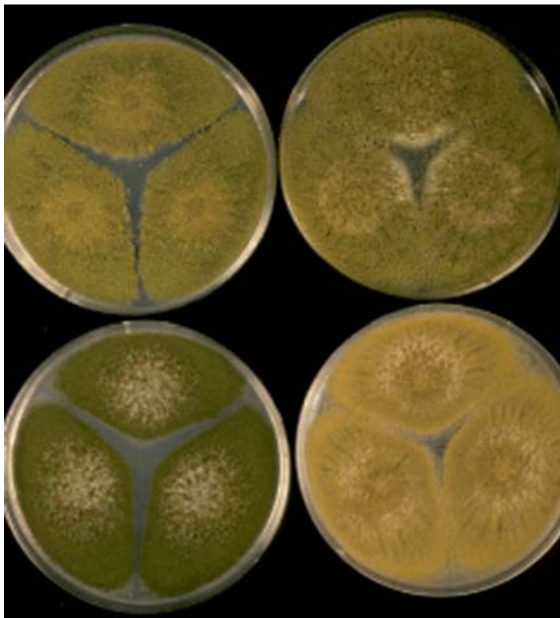

Fig. 1 Colonies of aflatoxin-producing fungi in *Aspergillus* section *Flavi* on Czapek yeast agar after 14 days at 25°C. Clockwise from the *upper left* are *A. flavus*, *A. nomius*, the O strain of *A. nomius*, and *A. parasiticus*

(30D–E5–7), *A. flavus* (29–30D–E5–7), and *A. parasiticus* (28–30D–E6–7). As the colonies of the O strain aged, the colony color shifted to olive brown (3–4D–E5–7 after 14 days and 4E5–6 after 21 days). The O strain produced an orange colony reverse on AFPA as did *A. flavus* and *A. parasiticus* but not the two isolates of the typical strain of *A. nomius*. The O strain produced the orange reverse on AFPA at 25 and 30°C but not at 37°C even though the fungus grew well.

All eight isolates of the O strain produced dark brown to black and globose to subglobose sclerotia submerged in the medium but produced none on the surface (Fig. 2; Table 2). Among the other aflatoxin-producing fungi, only the S strain of *A. flavus* also produced submerged sclerotia, although over 95% of the sclerotia produced by these isolates were on the medium surface (Table 2). The submerged sclerotia produced by the O strain were substantially smaller and fewer in number than the sclerotia produced by the other aflatoxin-producing fungi except for *A. parasiticus* isolate ATCC1018, which did not produce any sclerotia (Table 2). The submerged sclerotia of the O strain developed slowly with very few observed at 8 days of incubation. The number of sclerotia produced continued to increase for 43 days (Fig. 3).

Microscopic Characteristics

The diameter of conidia differed little among the aflatoxin-producing fungi. However, the roughness of the conidial walls did differ with the walls for the O strain and the typical strain of *A. nomius* being intermediate between the smooth walls of *A. flavus* and the distinctly rough walls of *A. parasiticus*. All isolates of the O strain produced more uniseriate heads than biseriate ones, similar to *A. parasiticus*. The percentage of uniseriate heads ranged from 75 to 100%, depending on isolate. Similarly, for *A. parasiticus* the percentage of uniseriate heads ranged from 50

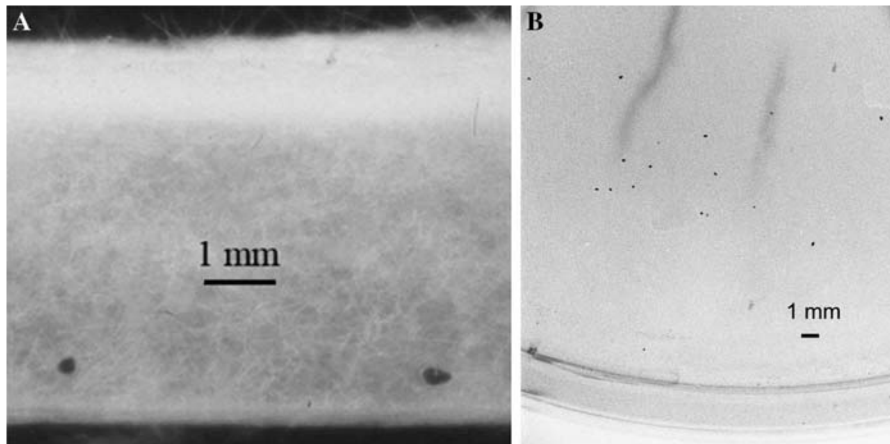

Fig. 2 Sclerotia of the O strain of *Aspergillus nomius*. **a** Cross section of agar medium showing two submerged sclerotia. **b** Reverse of Petri dish showing sclerotia submerged in the agar medium

Table 2 Characteristics of sclerotia produced by fungi in *Aspergillus* section *Flavi* in Czapeks–Dox agar after 30 days at 31°C

Species	Isolate	Number of sclerotia/cm[2a]		Length (mm)[b]
		On surface	Submerged	
O strain[c]	ATCC201127	0	0.25 ± 0.09	0.17 ± 0.03
	ATCC208927	0	0.36 ± 0.08	0.17 ± 0.04
A. nomius	ATCC15546[d]	210 ± 8	0	0.87 ± 0.04
	ATCC96015	55 ± 22	0	2.31 ± 0.10
A. flavus strain L	A228	1.5 ± 0.4	0	0.96 ± 0.12
	ATCC16883[d]	0.75 ± 0.04	0	0.92 ± 0.07
A. flavus strain S	A11611	194 ± 29	6 ± 0.3	0.37 ± 0.02
	AF42	150 ± 15	0.25 ± 0.09	0.38 ± 0.07
A. parasiticus	A240	0.49 ± 0.17	0	0.62 ± 0.12
	ATCC1018[d]	0	0	ND[e]

[a] Mean of three replicates ± SD

[b] Average length along the longest axis of the 20 longest sclerotia per plate ± SD

[c] For six other isolates of the O strain tested, no sclerotia were produced on the medium surface, from 0.13 to 0.75 sclerotia/cm^2 were submerged, and the sclerotia ranged from 0.12 to 0.21 mm in length, depending on the isolate

[d] Ex-type

[e] None detected

to 90%, depending on isolate. In contrast, both the typical strain of *A. nomius* (0–25% uniseriate heads) and *A. flavus* (0–40% uniseriate heads) predominantly produced biseriate heads. Conidiophores of the O strain were variable in length, typically 700–2,000 μm.

Effect of Temperature

The O strain grew faster in media at 15°C than the other aflatoxin-producing fungi including the typical strain of *A. nomius* (Table 3). At 42°C, however, the O strain grew poorly, which was similar to the growth of the typical strain of *A. nomius* and *A. parasiticus* but substantially less than the growth of *A. flavus* (Table 3). Nevertheless, at 30 and 37°C, the O strain grew very well having colony diameters greater than 80 mm after 7 days, which was similar to the other aflatoxin-producing fungi tested. None of the aflatoxin-producing fungi tested grew at 10°C. Among the temperatures tested, all the O strain

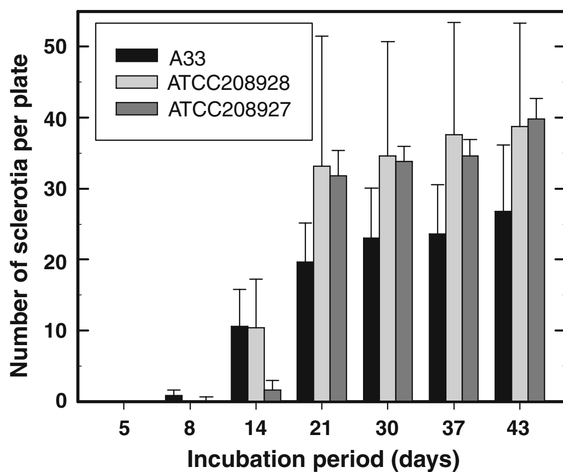

Fig. 3 Production of submerged sclerotia in agar medium (in 100 × 15 mm Petri dishes) by three isolates of the O strain of *Aspergillus nomius* during incubation at 31°C. Each data point is the average of five replicates. *Bar* SD

Table 3 Effect of temperature on fungal growth of aflatoxin-producing fungi in *Aspergillus* section *Flavi* in Czapek solution agar after 7 days

Species	Isolate	Colony diameter (mm)[a]	
		15°C[b]	42°C
O strain[c]	ATCC201127	24.3a	4.5de
	ATCC208927	24.3a	2.3f
A. nomius	ATCC15546[d]	20.3b	2.8ef
	ATCC96015	14.2e	4.7d
A. flavus	A228	17.5d	26.2a
	ATCC16883[d]	17.0d	23.5b
A. parasiticus	A240	19.7c	12.8c
	ATCC1018[d]	19.7c	4.7d

[a] At 30 and 37°C, the colony diameters were greater than 80 mm after 7 days for all isolates tested

[b] Numbers followed by the same letter are not significantly different ($P = 0.05$) by pairwise comparisons using Fisher's LSD

[c] For five other isolates tested, the individual means ranged from 23.2 to 26.5 and from 1.5 to 5.7 mm at 15°C and 42°C respectively

[d] Ex-type

isolates and other aflatoxin-producing fungi grew fastest at 30°C.

Mycotoxin Production

The O strain produced aflatoxins in A&M liquid medium, glucose yeast agar medium, and pistachio nuts (Tables 4, 5). In these substrates, the O strain typically produced substantially lower amounts of aflatoxin compared to the other aflatoxin-producing fungi, even though the O strain grew well. For example, the O strain grew approximately the same as the other aflatoxin-producing fungi in A&M liquid medium (Table 4). In addition, the O strain produced both the B and G aflatoxins, similar to the typical strain of *A. nomius* and *A. parasiticus*, whereas *A. flavus* produced only the B aflatoxins (Tables 4, 5). None of the isolates of the O strain, the typical strain of *A. nomius*, and *A. parasiticus* produced cyclopiazonic acid, whereas 90% of the isolates of *A. flavus* did (data not shown).

Discussion

An unusual characteristic of the O strain is that the sclerotia are submerged in the agar medium (Fig. 2). No other aflatoxin-producing fungus exclusively produced submerged sclerotia. The S strain of *A. flavus*, which is characterized by the production of abundant small sclerotia [3], did have a very small percentage of sclerotia submerged in the medium (Table 2). As far as we know, this is the first report of fungi (O strain and the S strain of *A. flavus*) in *Aspergillus* section *Flavi* producing submerged sclerotia. Most importantly, the sclerotia produced by the O strain differ substantially from those of the typical strain of *A. nomius*. The characteristic elongated sclerotia produced by *A. nomius* and initially described as indeterminate are important for distinguishing *A. nomius* from *A. flavus* [23, 25] and are not produced by the O strain. Instead, the O strain produces sclerotia that are substantially smaller and more spherical than the typical strain (Table 2). Furthermore, the sclerotia of the O strain are most abundant in older cultures (Fig. 3), thus it is important not to examine colonies too early. Although it is not clear why submerged sclerotia are being produced by the O strain, it could be related to an adaptation of some *Aspergillus* fungi to growing deep into substrates such as seeds [32] and could aid the fungus in survival or dispersal. Unfortunately, sclerotia of the O strain can easily be overlooked due to their small size, embedded nature, delayed production, and scarcity.

Although the O strain grew faster at 15°C in CZ than the other common aflatoxin-producing fungi, the

Table 4 Production of aflatoxins B1 and G1 in A&M liquid medium by the aflatoxin-producing species in *Aspergillus* section *Flavi*

Species	Isolate	Aflatoxins (ng)		Fungal mass (g)
		B1[a]	G1	
O strain	ATCC208927	152b	48b	0.986a
	ATCC208928	178b	21b	0.926a
A. nomius	ATCC15546[b]	259717a	87316a	0.864a
A. flavus	AF13	783972a	0c	0.967a
A. parasiticus	ATCC56775	1819346a	273201a	0.799a
	ATCC26691	1220143a	180092a	0.807a

[a] Numbers followed by the same letter are not significantly different ($P = 0.05$) by pairwise comparisons using Tukey's HSD test. Statistical analysis was performed on log-transformed data. Non transformed data presented for clarity

[b] Ex-type

Table 5 Production of aflatoxins B1 and G1 in glucose yeast agar medium and in pistachio nuts by the aflatoxin-producing species in *Aspergillus* section *Flavi*

Species	Isolate	Aflatoxin rating in medium[a]		Aflatoxin (ng/g) in pistachio nuts[b]	
		B1	G1	B1	G1
O strain[c]	ATCC201127	0.0c	0.0c	124b	254c
	ATCC208927	1.0bc	1.0b	0c	2d
	ATCC208928	0.0c	0.0c	166b	323c
A. nomius	ATCC15546[d]	4.0a	4.0a	402623a	881860ab
	ATCC96015	3.5a	4.0a	281967a	513097b
A. flavus	A228	4.0a	0.0c	737394a	0d
A. parasiticus	A240	1.0bc	1.0b	519397a	1712773a

[a] Numbers followed by the same letter are not significantly different ($P = 0.05$) by pairwise comparisons using Fisher's LSD. Ratings ranged from 0 to 4 with 4 representing the highest level of aflatoxin. The rating was done by comparing the brightness of fluorescent spots on TLC plates to that of three spots of known concentrations

[b] Numbers followed by the same letter are not significantly different ($P = 0.05$) by pairwise comparisons using Fisher's LSD. Statistical analysis was performed on log-transformed data. Non transformed data presented for clarity

[c] For four other isolates tested, the mean rating (depending on isolate) in medium ranged from 0.0 to 0.5 and from 0.0 to 1.0 for B1 and G1 respectively (mean ratings for B1 and G1 were 3.7 and 0.0 for eight other isolates of *A. flavus* and 2.8 and 2.9 for eight other isolates of *A. parasiticus*). For five other isolates of the O strain tested, the mean aflatoxin (ng/g) in pistachio nuts ranged from 13 to 666 and from 40 to 987 for B1 and G1 respectively

[d] Ex-type

O strain grew poorly at 42°C (Table 3). The typical strain of *A. nomius* also grows poorly at 42°C, showing less growth at this temperature than *A. flavus* and *A. parasiticus* [23] (Table 3). Growth differences at elevated temperatures such as 42°C have been useful in separating *A. nomius* from *A. flavus* [23].

The O strain produced both B and G aflatoxins (Tables 4, 5), which is similar to *A. parasiticus* and *A. nomius* but differs from *A. flavus* (typically does not produce the G aflatoxins) [10]. All isolates of the O strain produced only low amounts of aflatoxin in all substrates tested unlike the typical strain of *A. nomius*, which produced high levels of aflatoxin (Tables 4, 5). However, aflatoxin formation by the O strain in pistachio nuts still frequently exceeded by 100-fold the level of aflatoxins allowed in nuts imported to Europe, and as such low infection rates by O strain isolates could impact the marketability of crops. The relatively low amounts of aflatoxin produced by the O strain were not due to poor growth, because the O

strain grew well in these substrates. None of the O strain isolates produced cyclopiazonic acid, which is unlike *A. flavus* but similar to *A. parasiticus* [6].

Besides producing aflatoxin, the O strain has several characteristics in common with the other aflatoxin-producing fungi in *Aspergillus* section *Flavi*. The yellow–green heads and the black color of the sclerotia are characteristic of section *Flavi* [2, 15, 28]. In terms of microscopic features, these isolates of the O strain have the roughened conidiophore walls typical of section *Flavi*, and the sizes of the conidiophores and conidia are within the ranges found in section *Flavi* [2]. Also, the O strain produces an orange reverse in AFPA, which is similar to other aflatoxin-producing fungi [26], although many *A. nomius* isolates fail to produce the orange reverse [4]. An important reason for placing the O strain in *A. nomius* was that previous research using restriction fragment length polymorphism analysis indicated that the O strain was closely related to the ex-type of *A. nomius* [12]. Later research examining DNA sequences confirmed the close genetic similarity between the O strain and the ex-type of *A. nomius* [11, 24]. Using the sequence for the entire aflR gene and promoter regions (2,045 bases, Ehrlich et al. [11] as deposited in GenBank, National Center for Biotechnology Information), the O strain had 98 to 99% sequence identity with the ex-type of *A. nomius*. In contrast, the O strain had between 96 and 97% identity with the other two well-supported clades (11) of *A. nomius*. These results indicate that the O strain is too closely related to the ex-type of *A. nomius* to represent a distinct species. In addition, the O strain and the typical strain of *A. nomius* have other characteristics in common, such as relatively long stipes, similar appearing conidia, and production of the G aflatoxins but not cyclopiazonic acid.

The O strain differs from the other common aflatoxin-producing fungi in several ways (Table 1). For example, only the O strain typically produced sclerotia submerged in agar media. Good features for distinguishing species in *Aspergillus* section *Flavi* are colony color, conidial roughening, stipe or conidiophore length, and the presence of metulae resulting in biseriate heads [18, 22]. These features can also be used to distinguish the O strain from the other aflatoxin-producing fungi (Table 1). For example, the colony color of the O strain growing in CYA differs from the other common aflatoxin-producing species by being more yellowish in hue and closer to an olive color (Fig. 1). Another difference is that whereas *A. flavus* has smooth or slightly roughened conidia and *A. parasiticus* has rough conidia [19], the O strain has conidia with intermediate roughness. The O strain differs from the typical strain of *A. nomius* in sclerotium characteristics (size, shape, and location), the predominant type of head (uniseriate versus biseriate), colony color, growth at 15°C, and quantity of aflatoxins typically produced. It is not clear why the O strain is so different morphologically and physiologically from the typical strain of *A. nomius* as shown by our results, while being so similar genetically as has been shown by genetic studies [11, 12, 24].

The isolation of the O strain from orchards in California represents the first isolation of *A. nomius* in California. As far as the authors know, the typical strain of *A. nomius* has never been found in California. Besides occurring in pistachio and pecan orchards, more recently the O strain has been found in fig orchards in California. The O strain has been repeatedly isolated from a large area, represented by four counties (~200 km from the northernmost location to the southernmost location). The O strain is a previously undescribed fungus, which is important due to its aflatoxin production and of interest due to a combination of both close genetic relationship and distinguishing morphologies from the ex-type and other typical strains of *A. nomius*.

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