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Strain-Dependent Relationship Between Growth Rate and Hyphal Branching in Neurospora Crassa

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STRAIN-DEPENDENT RELATIONSHIP BETWEEN GROWTH RATE AND HYPHAL BRANCHING IN *NEUROSPORA CRASSA*

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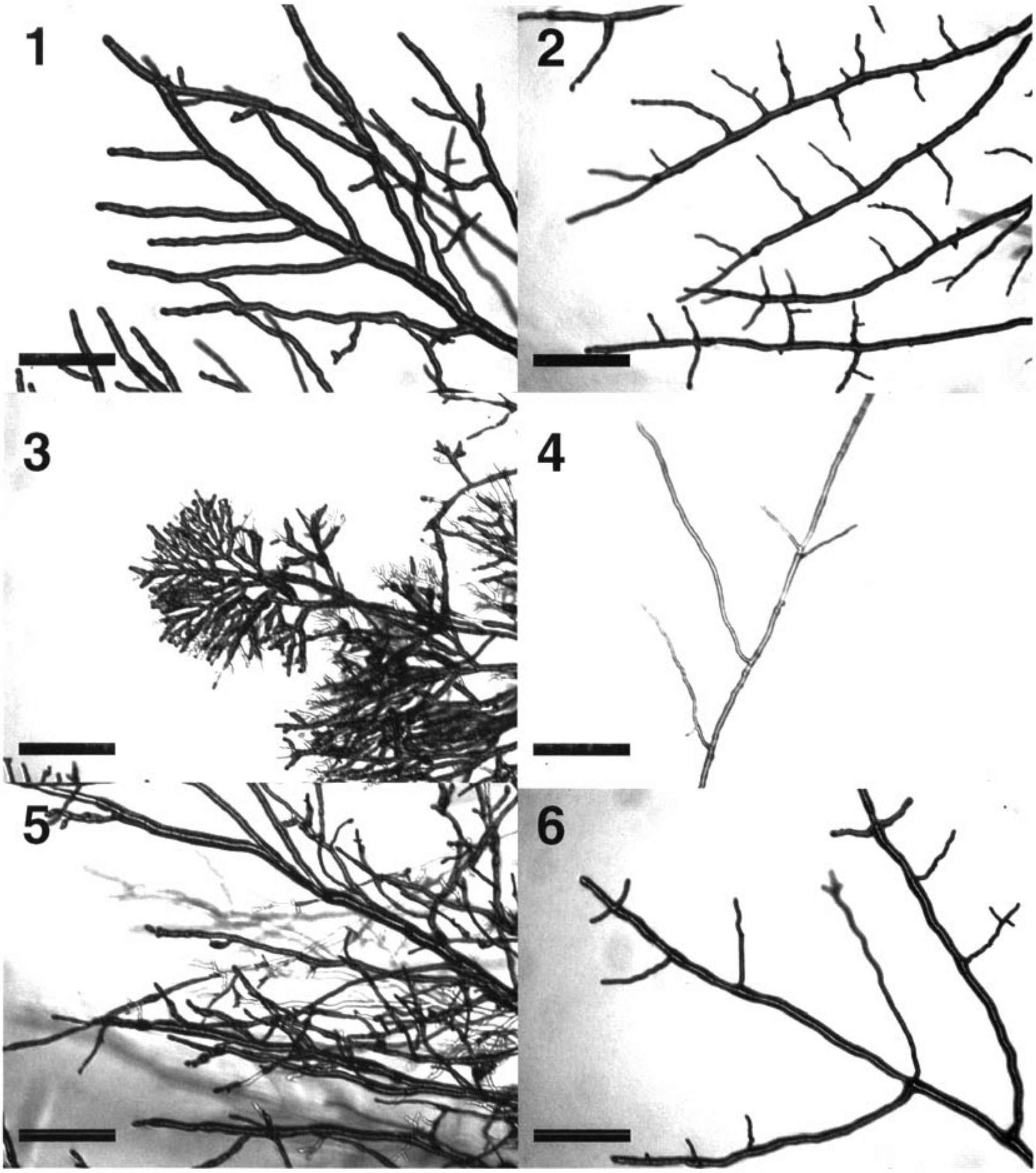
ABSTRACT. In a previous study of branch frequency in *Neurospora crassa* focused on the wild-type, no relationship between growth rate and the frequency of hyphal branching was observed. In subsequent experiments, it became clear that while this independence is valid for the wild-type and most mutant strains, it fails to hold for a subset of morphological mutants. This study distinguishes a subset of *Neurospora* morphological mutants for their morphological response to altered growth rate. Growth rates are altered using two different methods: reduced temperature and nutrient-deficient media. This should assure that the observed effect is not due to simple conditional mutations in the mutant strains examined. The observed effect provides an additional method for characterizing morphological mutants. It also provides support for models of branching in which control of branching is tightly linked to mechanisms of tip growth.

Keywords: *Neurospora crassa*, morphology, branching, hypha

Filamentous fungi grow by hyphal tip extension and by making new hyphal tips (branching). These two processes are fundamental aspects of fungal biology and have attracted much attention in research. Of the two processes, tip growth has been more extensively studied, leading to models for the cellular mechanisms involved (Bartnicki-Garcia 1973; Collinge & Trinci 1974; Heath 1990; Howard 1981; Riquelme & Bartnicki-Garcia 2004). There are a number of mathematical models for tip growth and morphogenesis (Hutchinson et al. 1980; Kotov & Reshitnikov 1990; Yang et al. 1992); but these do not attempt to explain branch initiation and usually assume branching to be a random event. A branch, once formed, must abide by the same growth mechanisms as the original apex. However, first a critical event presumably takes place that commits the cell to form a new branch.

The majority of models for branch initiation that have been proposed have tied branch initiation directly to the process of tip extension. Tip growth results from the polarized flow and exocytosis of "tip growth" vesicles at the apex of the growing tip (Heath, Gay & Greenwood 1971; Trinci 1974; Trinci 1978; Prosser & Trinci 1979; Bartnicki-Garcia, Hergert & Gierz 1989a; Bartnicki-Garcia, Hergert & Gierz 1989b; Bartnicki-Garcia 1990). In *N. crassa*, Zalokar (1959), studying regional variations in protein and RNA production, showed that material for tip extension can come from regions of the mycelium at least 12 mm from the colony margin. The importance of tip growth vesicles to branching is unclear, although branching symmetry studies have shown that the flow of material from within the colony is important in the determination of branching (Watters et al. 2000b; Watters 2006). It has been suggested that branching is induced when the concentration of tip growth vesicles reaches a critical density at the apex (Trinci 1974). Several studies have presented results that are consistent with this hypothesis (Katz et al. 1972; Trinci 1974; Watters & Griffiths 2001), but none demonstrate it definitively.

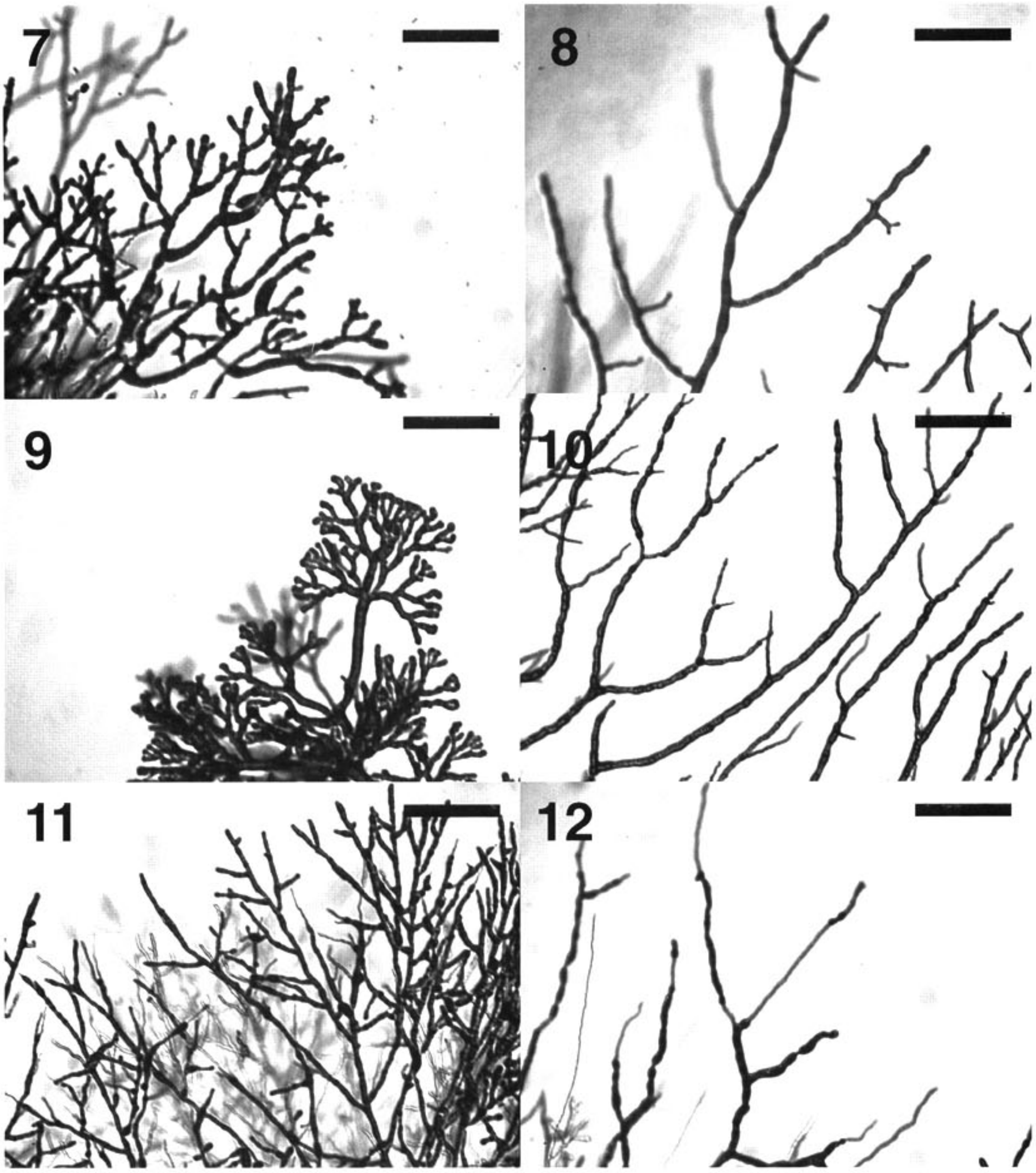
The strongest evidence to date relating branch initiation directly to tip growth has come from studies of Spitzenkörper behavior. The Spitzenkörper appears as a collection of tip growth vesicles located at actively growing hyphal tips, and has been shown to play a critical role in both tip growth and branching (Bartnicki-Garcia 2002; Riquelme & Bartnicki-Garcia 2004). Although the "critical density" proposition is consistent with observations of Spitzenkörper behavior during lateral branching events, Spitzenkörper behavior during apical branching (Riquelme & Bartnicki-Garcia



Figures 1–6.—Comparison of branching patterns of wild-type *Neurospora* and selected mutants at 33 °C and 10 °C. Scale bar is 200 μm. 1. *Neurospora* wild-type grown at 33 °C; 2. *Neurospora* wild-type grown at 10 °C; 3. Cum mutant at 33 °C; 4. Cum mutant at 10 °C; 5. Col-8 mutant at 33 °C; 6. Col-8 mutant at 10 °C.

2004) seems less supportive of this proposition. However, apical branching can be specifically induced by environmental insults (Watters et al. 2000a), genetic mutations (Scott 1976; Perkins et al. 2001) and cytoplasmic contractions (Riquelme & Bartnicki-Garcia 2004). These observations combined, argue that apical branches are induced via a unique mechanism and thus might be expected to give different results than lateral branches.

This proposed relationship between tip growth and branching might predict that the frequency of branching would vary with any variability in the rate of tip growth. This was previously tested (Watters et al. 2000a) by growing wild-type *Neurospora* at different



Figures 7–12.—Comparison of branching patterns of selected mutants at 33 °C and 10 °C. Scale bar is 200 μm. 7. Of mutant at 33 °C; 8. Pf mutant at 10 °C; 9. Sf mutant at 33 °C; 10. Sf mutant at 10 °C; 11. Ta mutant at 33 °C; 12. Ta mutant at 10 °C.

temperatures in order to control the growth rate. Branch periodicity was then measured under these various rates. Under these conditions, no relationship was observed between growth rate and the frequency of branching. This result was not consistent with the concept that branching was directly tip growth dependent. When this relationship was retested using a small number of morphological mutants (Watters & Griffiths 2001), a response was observed. In the strains thus responding, branching occurred less frequently at reduced growth rates. These results suggested that tip growth and branching might indeed be connected, but that this connection was masked in the wild-type. It remained possible that the

effects observed for the mutants were due to a previously undescribed temperature sensitive mutation. It was also unclear if the observed effect was strain specific or a general feature of tight-branching morphological mutants.

When the independence of tip growth and branching was explored further by shifting growth rates (via temperature shifts) during growth, a temporary shift in branching behavior was observed (Watters et al. 2000a; Watters & Griffiths 2001). The observation of a response during changing conditions argues that tip growth and branching are indeed connected. The lack of a response to steady-state growth conditions argued strongly that branching was subject to homeostatic controls that tended to negate the effects of the growth rate differences, resulting in very consistent branching patterns in wild-type *Neurospora*.

In the present work, this presumptive relationship is examined further by studying branching in a broad spectrum of known *Neurospora* morphological mutants under conditions that alter the rate of tip-extension. It expands on the previous report (Watters & Griffiths 2001) by increasing the number of morphological mutants examined from 3 to 40, as well as reducing growth rate by two different methods to eliminate the possibility of previously undetected conditional phenotypes of these mutations. The results presented below show that most morphological mutants tested, like the wild-type, do not appear to alter branching in response to different growth rates. A select group of mutants clearly branch less often under conditions of slow growth. It is reasonable to suggest that the mutants so highlighted represent mutations in the previously proposed homeostatic control system.

METHODS

Strains and media.—Neurospora mutant strains subjected to study were: wild-type (Oak Ridge), adh, bn, col-1, col-2, col-8, col-9, col-12, col-16, col-17, col-18, cpt, cr-1, cr-2, cr-4, cum, del, do, dot, fr, ipa, lp, med, mo-1, pe, pf, pi, pk, pl, rg-1, ro-1, sf, sh, sn, so, spco-4, spco-10, spco-12, ta, ti, vel. All *Neurospora* strains used in this study were obtained via the Fungal Genetics Stock Center (McCluskey 2003). Media and culturing procedures were those described in Davis & deSerres (1970). Growth described as being on "minimal" was on Vogel's minimal medium (Davis & deSerres 1970) whereas growth on "agar" was on plates containing 2% agar without any added nutrients or salts.

Examination of branching.—Photographs of growth at 33 °C were made following 24 h incubation. Photographs of growth at 10 °C were made following incubation for 7 days.

RESULTS

In order to probe the previously proposed homeostatic control system (Watters et al. 2000a; Watters & Griffiths 2001) further, the hyphal growth patterns of a number of previously identified morphological mutants were examined for the relationship between branching and tip growth. Reduced growth rates were induced by culturing strains under three different conditions: minimal at 33 °C, minimal at 10 °C and agar at 33 °C. Slowing growth using alternatively reduced temperature and reduced nutrient media assured that any consistently observed effects were not the result of previously unidentified conditional (i.e., temperature sensitive) phenotypes among these mutations.

The majority of mutants tested failed to show any clear relationship between branching and the rate of tip growth as determined by visual comparison. These strains (adh, bn, col-1, col-2, col-9, col-12, col-17, cpt, cr-1, do, fr, ipa, lp, med, pi, pl, rg-1, sh, sn, so, spco-4, spco-10, spco-12, ti) showed similar branching patterns under all three culture conditions. Growth of the Oak Ridge wild-type strain on minimal at 33 °C and 10 °C is shown in Figs. 1 & 2; others data not shown.

A select group of mutants (col-8, col-16, col-18, cr-2, cr-4, cum, del, dot, mo-1, pe, pf, pk, ro-1, sf, ta, vel) showed obviously reduced branching under conditions of slow growth rate. Photos of representative mutants on minimal at 33 °C and 10 °C are shown in Figs. 3–12.

DISCUSSION

Previous work in Neurospora (Watters et al. 2000a; Watters & Griffiths 2001) has suggested that branch initiation is controlled by a homeostatic system that compensates for tip growth rate.

It follows that it should be possible to generate mutations in the system responsible for maintaining the homeostatic set point.

These mutants would be expected to fail in their regulation of branching in such a way that exposes the dependence of branch frequency on growth rate.

The results presented above show the results of a survey of several previously identified morphological mutants of *Neurospora* for the phenotype expected for a mutant in the proposed homeostatic system.

The majority of strains tested failed to respond, showing similar branch frequencies under all growth conditions (*Neurospora* wild-type shown Fig. 1 vs. Fig. 2; others data not shown). This observation matches the previous data (Watters et al. 2000a) for the wild-type suggesting that the proposed homeostatic system is unaffected in these strains. Since this classification was determined qualitatively by visual observation, it remains possible that some of the non-responding strains would reveal modest responses to slow growth rate which escaped detection in this survey.

A subset of morphological mutants (col-8, col-16, col-18, cr-2, cr-4, cum, del, dot, mo-1, pe, pf, pk, ro-1, sf, ta, vel), shows a clear change in their branching frequency (Figs. 3–12). The responding mutants show a consistent reaction to slow growth. Under conditions that slow the rate of tip growth, these strains branch less frequently.

The observation of effects on branching under both low temperature and low nutrient conditions demonstrate that the observed effect is not due to a previously undiscovered conditional phenotype in these mutations. By eliminating simple conditional phenotypes, we can be more confident that the observed effects are the result of the shifts in growth rate and not the result of the conditions used to alter the growth rate.

The observed effect can be used to further classify both existing and novel Neurospora morphological mutants. Most of the known morphological mutants have been described solely on the basis of their behavior on Vogel's minimal medium under standard incubation conditions. The results presented here demonstrate that the phenotype of these strains can vary considerably under different growth conditions. Thus, the morphology of mutants under different conditions cannot necessarily be predicted based on their behavior under standard incubation conditions alone.

These results support models for the control of morphology that closely tie branching to tip growth. The mutants that respond most clearly to variations in growth rate serve to expose a relationship between these two features. This relationship remains masked in the wild-type. The mutants thus identified may prove useful in the further exploration of the relationship between tip growth and branching.

It further seems likely that the mutants that show a morphological response to growth rate are more likely to be involved in the homeostatic system proposed to govern branch initiation.

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