Lecture

Disruption of Mitosis

1. General Information

Mitosis is the process by which eukaryotic cell nuclei divide to give two daughter nuclei with identical and complete sets of chromosomes. The term mitosis, however, is commonly used to describe the entire process of cell division.

This process mainly occurs in the meristematic tissues and is absolutely necessary for plant growth. Herbicides with this mode of action inhibit the formation or function of the spindle apparatus, a framework of proteins that allows segregation of the daughter chromosomes in the division process. For one herbicide with this mode of action the specific target site is unknown.

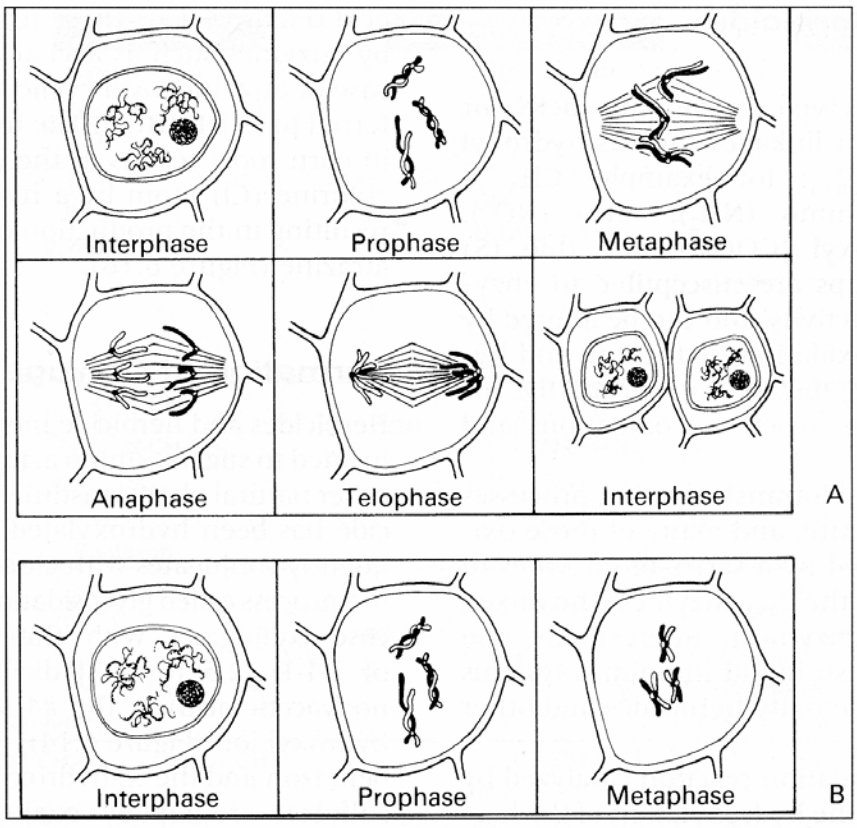


Figure 6.13 (A) Normal mitotic sequence and (B) effect of a dinitroaniline herbicide on mitotic sequence in root cells. (F. D. Hess, Novartis Crop Protection, Inc.)

2. Mode of Action

Microtubule Assembly Inhibitors (Interference with Spindle Fiber Formation)

For herbicides with this mode of action, the prophase sequence is normal, but without the spindle apparatus, chromosomes are unable to move into the metaphase configuration and daughter chromosomes cannot migrate to their respective poles.

Herbicides representing the dinitroaniline family bind to tubulin, the major microtubule protein. The herbicide-tubulin complex inhibits polymerization of microtubules at the growing end of the tubule but has no effect on depolymerization of the tubule on the other end. This leads to loss of microtubules.

In contrast, the herbicides dithiopyr and pronamide do not bind to tubulin but appear to bind to another protein that may be a microtubule associated protein. The end result is shortened microtubules that cannot form the spindle fibers needed for mitosis. In both cases, after a time in the prophase state, chromosomes coalesce in the middle of the cell, cell division ceases, and growth stops.

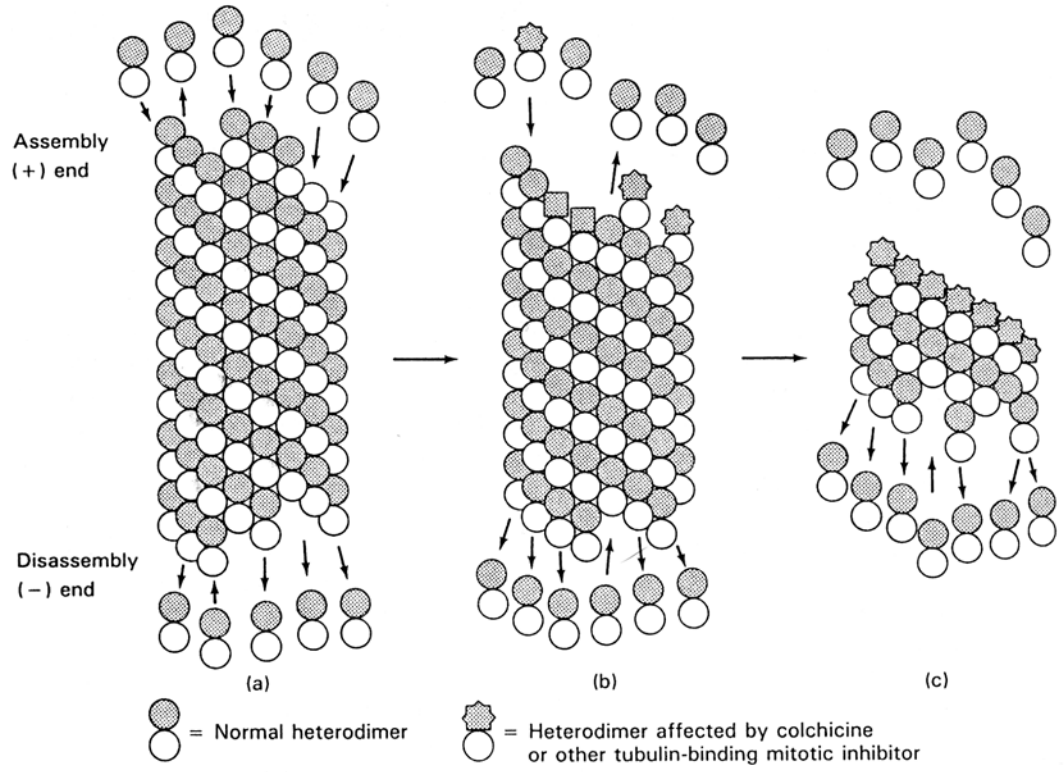


Figure 10.1 Assembly and disassembly of tubulin heterodimers into microtubules. Heterodimers from the free pool normally preferentially assemble into the assembly (+) end of the microtubule (A). When the heterodimer is altered by compounds such as colchicine or certain dinitroaniline herbicides, assembly at this end is prevented; however, disassembly is not prevented (B and C).

Source: Physiology of Herbicide Action. M.D. Devine, S.O. Devine, C. Fedtke. 1993. Prentice Hall, NJ.

Disruption of Mitosis (Interference with Spindle Fiber Function)

Herbicides in this group were at one time used for weed control in wheat, alfalfa, and other crops, but have been discontinued and are no longer labeled. These herbicides disrupt mitosis by interfering with spindle fiber function. Their mode of action is included only to provide a contrast with the microtubule assembly inhibitors that interfere with spindle fiber formation.

For herbicides with this mode of action the prophase sequence is normal. Spindle fiber formation occurs but spindle fibers cannot function properly, i.e. they cannot separate the daughter chromosomes in the division process. Specifically, these herbicides cause chromosome movement during anaphase toward three or more foci, rather than two foci of a normal anaphase. After this multipolar division, the nuclear membranes re-form around the micronuclei, resulting in highly branched and oddly shaped phragmoplasts.

The abnormal phragmoplasts organize abnormal and irregularly shaped cell walls. Apparently, the carbamate herbicides disrupt the spindle microtubule organizing centers, fragmenting then throughout the cell. Chromosomes clump in several areas of the cell rather than along the single metaphase plate. Mitosis is disrupted and growth increases.

3. Site of Action

Microtubule Assembly Inhibitors (Interference with Spindle Fiber Formation)

Herbicides inhibiting the formation of the spindle apparatus act at the microtubules, which originate from the endoplasmic reticulum and the nuclear membrane.

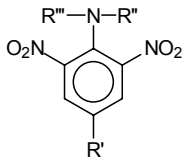
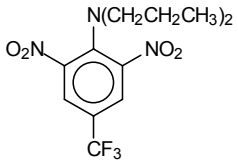
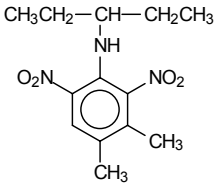
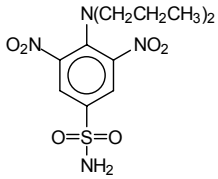
Disruption of Mitosis (Interference with Spindle Fiber Function)

The molecular site of action is not known.

4. Symptoms (all herbicides that disrupt mitosis)

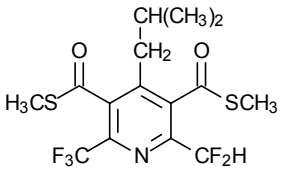
- germination is not inhibited but seedlings fail to emerge from soil
- root swelling and lateral and secondary root development inhibited
- clubbed roots or stubby roots
- swelling of hypocotyls of broadleaf crops and stem bases of grasses
- some grasses may turn purple at the base

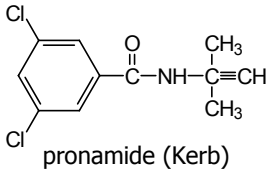
5. Herbicide Families – Disruption of Mitosis (Interference with Spindle Fiber Formation)

<table>
<tr><td colspan="3">Dinitroanilines (DNA's)</td></tr>
<tr><td>Base Structure</td><td>  </td><td>Eli Lilly Company discovered this family in 1960; referred to as "yellow herbicides" since they are yellow-orange crystalline solids; have low water solubility, susceptible to volatility and photodegradation; base is an aniline ring structure (benzene ring with an NH_2 group attached) with a nitro groups (NO_2) attached to the 2 and 6- C's, and substitution at the 4-C</td></tr>
<tr><td>Examples</td><td colspan="2"> 
trifluralin (Treflan/Trilin, etc.)
 
pendimethalin (Prowl/Pentagon)
 
oryzalin (Surflan)</td></tr>
<tr><td>Others</td><td colspan="2">benefin (Balan) – used in tobacco, lettuce, etc.
ethalfluralin (Sonalan) – used in squash, pumpkins, etc.</td></tr>
<tr><td>Metabolism</td><td colspan="2">plant: dealkylation, NO_2 reduction
soil: microbial
half-life – trifluralin 45 d; pendimethalin 44 d; oryzalin 20 d</td></tr>
<tr><td>Absorption & Translocation</td><td colspan="2">absorbed by emerging shoots (grass coleoptiles, broadleaf hypocotyls or epicotyls) and roots; have little to no POST activity
translocation limited and not necessary because of mode of action</td></tr>
<tr><td>Selectivity</td><td colspan="2">selective – herbicide placement that avoids contact with roots of desired plants is primary factor; taproot growth much less affected than lateral roots
these herbicides control annual grasses and small-seeded broadleafs</td></tr>
<tr><td>Herbicide Use</td><td colspan="2">trifluralin – PPI (incorporation within 24 h; due to slight volatility and photodegradation); alfalfa, dry beans, carrots, celery, cole crops, okra, peas, peppers, sunflowers, trees, lima beans, snap beans, cotton, soybeans, sugarcane, ornamental shrubs, ground covers; 2X rate for johnsongrass control; prickly sida release and cheesecloth story

pendimethalin – PRE, early POST in field and sweet corn; POST in grain sorghum and rice; PPI/PRE in soybeans, cotton, potatoes, sugarcane, beans, peas, fruit and nut crops, turf

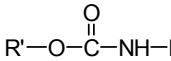
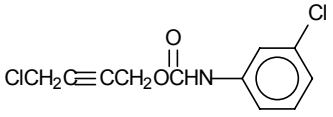
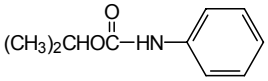
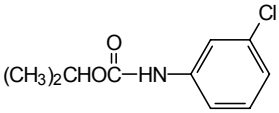
oryzalin – PRE in fruit and nut crops, vineyards, landscape nurseries, right-of-ways, Christmas tree plantings</td></tr>
</table>

Pyridazines		
Example	 dithiopyr (Dimension)	sometimes called a substituted pyridine (note pyridine ring), but mode of action is not that of an auxin growth regulator herbicide like other pyridine herbicides discussed previously; dithiopyr does not bind to tubulin like the DNA's
Metabolism	plant: not available soil: microbial half-life – 17 d	
Absorption & Translocation	absorbed by roots and to some degree by foliage of susceptible plants limited translocation	
Selectivity	selective but factors are unknown	
Herbicide Use	controls annual grasses and small-seeded broadleafs PRE or POST in direct seeded rice, established cool season and warm season turf (crabgrasses); PRE ornamentals, trees, and other perennial crops	

Amides		
Example	 pronamide (Kerb)	amide base structure; another acid amide herbicide (propanil; pg 59) has a different mode of action; also propanil is applied POST whereas pronamide is applied PRE; pronamide does not bind to tubulin like the DNA's
Metabolism	plant: not available soil: microbial half-life – 60 d	
Absorption & Translocation	absorbed by roots and translocated in apoplast	

Selectivity	selective but mechanism is not reported
Herbicide Use	controls grasses and broadleafs PRE in artichokes, blackberries, boysenberries, raspberries, blueberries, established legumes, rhubarb, woody ornamentals, nursery stock, Christmas trees, apples, cherries, nectarines, peaches, pear, plum, grapes, turf Restricted use pesticide

6. Herbicide Family – Disruption of Mitosis (Interference with Spindle Function)

Carbamates		
Base Structure		herbicides in this family have been discontinued and are no longer registered in any crop; they have been included only to contrast the specific mechanisms of action for disruption of mitosis; compare with phenyl carbamates
Examples	 barban (Carbyne)  propham (Chem Hoe)  chlorpropham (Furloe/Sprout Nip)	

7. Mode of Action

<u>Disruption of Mitosis with Unknown Target Site</u>

The herbicides in this class consist of only one example, DCPA. Very little is known about this herbicide and its precise mode of action has yet to be determined.

It is suspected that DCPA inhibits mitosis by affecting specialized microtubule assemblies called phragmoplasts. The phragmoplasts are assemblies made of dissociated spindle units that are involved in cytokinesis, the process of cell partitioning into two daughter cells.

Some sources classify this herbicide as a cell wall inhibitor because no cell wall is formed between the daughter cells (cell plate), resulting in cells with more than one nuclei. DCPA, like the dinitroanilines, stop mitosis at the metaphase stage.

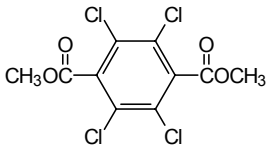
8. Site of Action (Unknown Target Site)

DCPA most likely acts on the phragmoplast assembly of microtubules in dividing cells.

9. Symptoms (Unknown Target Site)

Symptoms of DCPA injury are similar to that of the dinitroanilines, pyridazines, and amides. Differences of herbicidal effects are usually only evident at the cellular level. DCPA, unlike other mitotic inhibitors, often results in multinucleate cells.

10. Herbicide Families (Unknown Target Site)

Non-Family		
Example	 DCPA (Dacthal)	sometimes referred to as phthalic acid family or benzene dicarboxylic acids
Metabolism	plant: not available soil: microbial half-life – 60-100 d	
Absorption & Translocation	absorbed by roots and shoots translocation minimal	
Selectivity	mechanism not clear	
Herbicide Use	controls grasses and small seeded broadleafs, but less effective than others used PRE in established turf or in newly seeded turf, ornamentals, *Brassica* crops, beans, melons, cucumbers, squash, garlic, greens, horseradish, onions, potatoes, yams, tomatoes, eggplant, peppers, strawberries, cotton	

11. General Comments

Trifluralin, one of the better known DNA's, was discovered by Q.F. Soper in 1960. It is still used today in many crops as a preemergence incorporated treatment because of its affordability. Pendimethalin was introduced more recently in 1974 by American Cyanamid (now BASF) and is used on most row crops grown in the U.S. Asulam, a phenyl carbamate herbicide, has mitotic inhibition as its secondary mode of action.

In contrast to pronamide (Kerb), the amide herbicide propanil (Stam) is an inhibitor of photosynthesis at Photosystem II, and the amide herbicides metolachlor (Dual), alachlor (Lasso), napronamide (Devrinol), and others are seedling root/shoot inhibitors (see handout 15).

Unlike the herbicides barban, propham, and chlorpropham discussed in this handout, the carbamate herbicide asulam (Asulox/Asulam) is involved in folic acid synthesis (DHP synthase inhibitor).

12. References

Ahrens, W. Herbicide Handbook, seventh edition. 1994. Weed Science Society of America,

Anderson, W.P. Weed Science – Principles and Applications, third edition. 1996. West Publishing, NY.

Devine, M.D., S.O. Duke, and C. Fedtke. Physiology of Herbicide Action. 1993. Prentice Hall, NJ.

Ross, M.A. and C.A. Lembi. Applied Weed Science, second edition. 1999. Prentice Hall, NJ.