A NOTE ON THE PROPER APPLICATION OF ALPHA-ALPHA DIPYRIDYL TEST STRIPS FOR HYDRIC SOIL IDENTIFICATION

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INTRODUCTION

The application of α,α'-dipyridyl dye (pronounced alpha, alpha di-peeri-dill) provides a reliable and defensible mechanism for documenting the presence of reduced iron in support of hydric soil identification and wetland delineation. The dye has proven particularly useful for identifying naturally problematic, altered, or disturbed hydric soils. The proper application of paper test strips embedded with α,α'-dipyridyl dye further promotes the use of this technique to improve wetland delineation and management. This note provides practitioner recommendations for applying, documenting, and interpreting α,α'-dipyridyl dye test strips.

In practice the α,α'-dipyridyl dye test strip is applied to naturally moist or wet soil and, if a colorimetric reaction occurs producing a red or pink color, the presence of reduced iron (and anaerobic conditions) is confirmed, and the definition of a hydric soil is met (Figure 1). Notably, application of the dye to a dry soil will not result in a color change and is not indicative of the presence or absence of hydric soils. When reduced iron is present in soil solution, the α,α'-dipyridyl dye chemically binds with the soluble iron to produce the reddish or pink reaction via chemical complexation. For many years α,α'-dipyridyl dye was applied to the soil as a liquid. More recently, paper test strips impregnated with α,α'-dipyridyl became available, representing an affordable (~$0.25 USD per strip), easy approach to dye application that reduces barriers to practitioner use. These commercial products are most often advertised as 'dipyridyl paper' and are available from a variety of online chemical manufacturing and commercial retailers. The paper test strips were developed to test for the presence of Fe^{2+} in industrial manufacturing context but have proven effective in the wetland sciences.

Figure 1. Example of α,α'-dipyridyl dye paper test strip. Note that the positive reaction is clearly visible both on the soil surface and on the paper test strip and that the reaction occurs across >60% of the soil layer. (Photo: USACE staff)

Berkowitz et al. (2017) demonstrated that α,α'-dipyridyl dye liquid and paper test strip formulations delivered similar results, exhibited similar detection limits, and worked well across a broad range of soils. Wetland practitioners display a preference for the paper test strips over the liquid dye formulation because 1) no laboratory apparatus or preparation is required, 2) no special field equipment (e.g., spray bottle, dropper) is required, and 3) evidence suggests that the paper test strips may be more robust for typical field applications (Berkowitz et al. 2017).

APPLYING α,α'-DIPYRIDYL DYE TEST STRIPS

In order for α,α'-dipyridyl dye test strips to be used, the soil must be naturally saturated (wet or moist). Users should not apply the dye to dry soil or attempt to wet the soil prior to application. The α,α'-dipyridyl dye test strip should always be applied to the soil sample as soon as it is excavated from the ground. When doing so, a ped of soil should be opened by hand to expose its interior. Care must be taken to avoid placing the dye on surfaces that have come into contact with metal sampling tools including shovels, soil knives, augers, or other implements that may contain iron. Press the paper strip firmly onto the soil surface to ensure maximum contact with the soil solution. Enclosing the test strip between freshly exposed ped faces often provides an effective technique.

Notably, some of the commercially available α,α'-dipyridyl dye impregnated test paper kits include instructions to "Apply a drop of weak acid (>pH 2) to the test paper. In the presence of Fe(II)-ions a red spot appears, or in the presence of small quantities of Fe(II)-ions a red ring." However, application of acidic compounds should be avoided during wetland evaluations, because the addition of acid has the potential to dissolve oxidized iron compounds in the soil and generate false positive results. Recall that the paper test strips were developed for industrial manufacturing applications unrelated to hydric soil evaluations, thus these instructions should be ignored when using α,α'-dipyridyl dye test strips in wetlands.

To illustrate the problem with applying acid solutions during α,α'-dipyridyl dye application, triplicate benchtop studies were performed using two soils (Table 1). The study utilized α,α'-dipyridyl dye test strips in the absence of acid and following the addition of 0.1N Hydrochloric (HCl) acid. Note that the addition of weak acid induced a false positive dye reaction in both soils. This observation occurs because acid addition decreases pH and increases iron solubility (Hem and Cropper 1962), allowing Fe^{2+} to enter soil solution and complex with the α,α'-dipyridyl dye to generate a false positive color response.

Soil Order	Oxisol	Vertisol
pH	7.97	7.39
USDA Classification	Moderately alkaline	Neutral/Slighly alkaline
Reaction without Acid Addition	Negative	Negative
Reaction following Acid Addition	False positive	False positive

Table 1. Effect of acid addition on α,α'-dipyridyl dye response.

INTERPRETING α,α'-DIPYRIDYL DYE TEST STRIP RESULTS

A positive reaction to α,α'-dipyridyl dye test strips is defined in the Hydric Soil Technical Standard as: "A positive reaction must occur within 60% or more of a specific soil layer in at least two of three soil samples. The positive dye reaction must occur within a 10-cm-thick (4 in.) layer in the upper 30 cm (12 in.) for most soils, a 6.25-cm-thick (2.5 in.) layer within the upper 12.5 cm (5 in.) in sandy textured soils, or a 5-cm-thick (2 in.) layer within the upper 10 cm (4 in.) in soils that inundate by flooding or ponding" (Berkowitz et al. 2021).

Following application, careful evaluation of the α,α'-dipyridyl dye test strip and the exposed soil is required. In soils subject to prolonged periods of saturation that contain ample sources of Fe and organic matter, the dye reaction is often nearly instantaneous and readily observable upon test strip application. However, soils with low Fe content, low organic matter content, dark soils, or soils experiencing short wetland hydroperiods often display faint responses that require careful examination (Figure 2). Positive reactions to the dye are typically visible within thirty seconds to one minute of application but may take longer in soils with low amounts of ferrous iron, or when cold temperatures are present. Rabenhorst et al. (2021) reported that iron reduction rates may decrease when temperatures fall below ~10 °C (50 °F). This may delay positive α,α'-dipyridyl dye reactions by a few minutes, however the authors of this note have observed positive dye reaction during cold temperatures, even in permafrost soils. Development of red or pink color over periods exceeding a few minutes (e.g., >5) should not be relied upon because the potential for photochemical complexation of the dye with organic compounds is possible, representing a potential false positive (Childs 1981). False positives also occur if soils or test strips are contaminated by shovels, augers, etc.

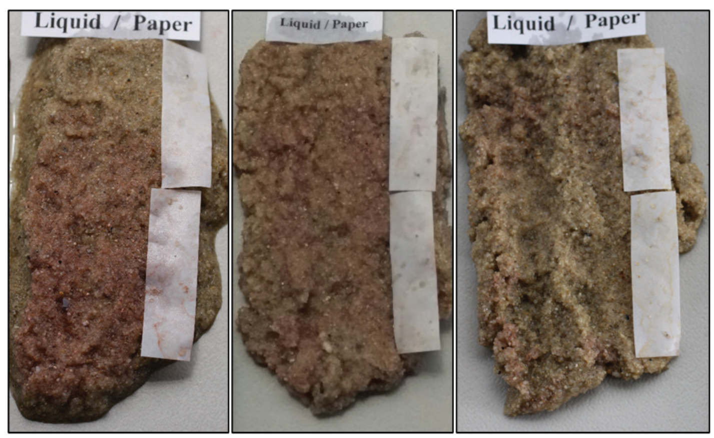

Figure 2. Examples of positive reactions to α,α'-dipyridyl dye exposed to both liquid (left side of peds) and paper test strip (right side of peds) formulations. Note the range of responses from clearly visible reactions in the left image to moderate responses in the center, and faint reaction in the right image. (Photo: USACE staff)

False negative reactions can also result in several scenarios, including application of the test strips to unsaturated soils, and soils saturated for an insufficient time period to reduce iron. Soils with low iron or organic matter content, including many sandy soils, can also fail to form a reaction. As a result, it is important for practitioners to recognize that a negative reaction to α,α'-dipyridyl dye does not indicate that hydric soils (or wetlands) are absent.

Previous studies identified the potential for α,α'-dipyridyl dye to degrade with exposure to light or heat (Childs 1981). In response, users are encouraged to store α,α'-dipyridyl dye test strips under dark and cool conditions to the extent possible. Operationally, practitioners report reliable results after keeping α,α'-dipyridyl dye paper test strips in field vests exposed to hot conditions, even across multiple field seasons. If questions arise related to the reliability of available α,α'-dipyridyl dye test strips, applying the strips to soils you know have remained saturated for long periods is recommended, or in areas where iron sheens associated with reduced iron are observed. Solutions of ferrous ammonium sulfate also document the reliability of α,α'-dipyridyl dye test strips (Berkowitz et al. 2017).

SUMMARY

Accurate determinations of the presence of hydric soils and wetlands require a comprehensive assessment of site conditions, soil morphology, and other factors. However, the proper use of α,α'-dipyridyl dye test strips provides a valuable tool to improve the management of hydric soil and wetland resources and is recommended for a variety of applications including wetland delineation, restoration, research, and public education and outreach. Additionally, α,α'-dipyridyl dye test strips can inform the management of difficult wetland delineation scenarios, including interpreting areas subject to naturally problematic situations (e.g., red or black parent materials, low levels of organic matter, high pH, and high chroma soils) and anthropogenically disturbed areas (e.g., fill material). When used with care, α,α'-dipyridyl dye test strips provide a valuable tool to quickly identify the presence of reduced iron and promotes the adoption of the dye application to improve the management of wetland resources.

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